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THE PULPLESS TOOTH FROM A BACTERIOLOGIC AND EXPERIMENTAL STANDPOINT*

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THE thoughtful clinician in evaluating the pulpless tooth as a factor in disease asks three questions: 1. How frequently is the pulpless tooth infected; how far can one translate radiographic evidence of infection into terms of bacteria? 2. Are the bacteria found in areas of dental infection able to produce disease; if so, with what frequency? 3. In individual cases what experimental proof is there that a focus of dental infection bears a causal relation to the patient's symptoms? The answers to these questions must constitute the scientific basis for determining the rôle that dental infection plays in the causation of chronic infectious disease. The problem is largely a bacteriologic one, yet it is surprising how little data is available concerning the points mentioned. There is not in the literature a single comprehensive bacteriologic study of dental infection by modern methods; relatively little work has been done to determine the pathogenicity of mouth streptococci by the correct technic; very little experimental proof concerning the causal relation of a focus to a disease in individual cases is available except that of Rosenow and coworkers and of Price.[6] I have attempted by cultures of dental infection and by certain animal experiments to obtain further facts in answer to the questions asked. This data is presented below.

1. THE BACTERIOLOGIC STATUS OF THE PULPLESS TOOTH

To determine, if possible, the exact bacteriologic status of the pulpless tooth, we have cultured the apices and periapical tissues of a large number of pulpless teeth. A quantitative technic has been employed by which the

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number of bacteria present in the tissues cultured can be determined. The extractions have all been done by Charles W. Keeling and Carl D. Lucas, whose cordial cooperation has made possible this study. The technic used in obtaining the culture material is as follows: The teeth are first thoroughly scrubbed off with gauze, and the gums are then painted with tincture of iodine followed by alcohol. The field of operation is packed off with sterile gauze and the tooth extracted with sterile forceps. The apex of the tooth is cut off with sterile forceps directly into a sterile tube containing about 1 c.c. of salt solution and a small amount of sand.

A. *B.*

Fig. 1.—Cultures from periapical dental infection in deep tubes of glucose brain broth agar. *A*, culture in which the growth is uniform throughout the tube; *B*, culture in which the organism grows only about three-fourths of the way to the top of the tube.

In making the bacteriologic cultures we have followed closely the technic of Rosenow. Each tooth has been cultured in deep tubes of glucose brain broth and glucose brain agar. These mediums afford all degrees of oxygen tension and are thus especially favorable for the growth of the streptococci found in chronic focal lesions. The technic used in the preparations of the mediums has been described in detail elsewhere.[1] The tube containing the root tip in the sterile sand and salt solution is well shaken to macerate the tissue on the tip of the tooth as completely as possible. The mediums are inoculated by pouring the salt solution containing the suspended tissue into a deep tube of glucose

brain agar which has been heated and allowed to cool to 40° C. The small amount of salt solution remaining in the tube is then poured into a tube of glucose brain broth. The inoculated tubes are incubated at 37° C. for twenty-four to forty-eight hours. The agar tube is employed only to determine the number of bacteria present. The appearance of such a positive culture is shown in Fig. 1. Smears are made from the broth tube to determine the type of organism and the broth culture is then used for animal inoculation.

In the statistics to be presented, I have included cultures of the incisors, cuspids and bicuspids only, since extraction of the molars without mouth contamination is often quite difficult. The different steps in the technic have been repeatedly checked bacteriologically to detect possible errors. As a further check on technic, I have constantly cultured vital teeth to determine the percentage of error.

TABLE I

RESULTS OF QUANTITATIVE CULTURES FROM PERIAPICAL DENTAL INFECTIONS

GROUP	NUMBER CULTURED	NUMBER SHOWING IN DEEP AGAR TUBE			NUMBER STERILE IN BROTH
		1 OR MORE COLONIES	10 OR MORE COLONIES	OVER 100 COLONIES	
Vital teeth	392	14%	5%	1%	46%
Pulpless teeth with negative radiograph	490	54%	44%	24%	18%
Pulpless teeth with positive radiograph	425	70%	63%	44%	9%
All pulpless teeth	915	61%	51%	33%	14%
Total	1307				

The results of the cultures made in the manner outlined of 1307 teeth are shown in Table I. I have not included in the series any teeth in which there was a question of contamination from saliva, lips, tongue or otherwise at the time of extraction. Likewise, I have excluded teeth showing pyorrhea. The teeth cultured are fairly equally divided between vital teeth, pulpless teeth with negative radiograph, and pulpless teeth with positive radiograph. Three hundred ninety-two vital teeth have been cultured. Of these 14 per cent have shown 1 or more colonies in a deep agar tube, 5 per cent 10 or more colonies and 1 per cent have had over 100 colonies. It seems reasonable to take 10 or more colonies as an arbitrary number as indicating sufficient infection to be of possible importance from a systemic standpoint. These results indicate also the percentage of error which must be allowed for interpreting the results in pulpless teeth. The positive cultures do not all represent, however, errors in technic. Some of the teeth considered as vital were probably pulpless, since all were not tested for vitality, and others had large cavities and might well have had infected pulps. Forty-six per cent of the broth cultures from these teeth were sterile.

Four hundred twenty-five pulpless teeth with positive radiograph are included. Of these 70 per cent showed 1 or more colonies in the deep agar tube, 63 per cent 10 or more colonies, and 44 per cent over 100 colonies in the deep tube. Nine per cent of the broth cultures in this group were sterile.

The significant findings here are the facts that 40 per cent of the teeth with positive radiographic evidence of infection showed less than 10 colonies and 9 per cent were sterile in broth. It is evident that in many cases the infection had run its course and healing had taken place so far as the presence of bacteria is concerned.

Four hundred ninety pulpless teeth with negative radiograph have been cultured. Fifty-four per cent of these have had 1 or more colonies in the deep agar tube, 44 per cent 10 or more colonies, and 24 per cent over 100 colonies; 18 per cent were sterile in broth. I realize that there may be much discussion and difference of opinion concerning what radiographic criteria should be employed in placing teeth in this group. We have taken a conservative attitude as to what is positive or negative. The striking thing here is the high percentage of positive cultures. The incidence of infection is almost as high as in those with a positive radiograph. Infection in the tooth with a negative radiograph is probably more serious from the systemic standpoint that when the radiograph is positive, since little resistance on the part of the body is indicated, and absorption is probably more rapid. The organisms recovered have been streptococci in pure culture or mixed with staphylococci. Only rarely have other organisms been encountered.

I should like to emphasize that these cultures so made, show only the bacteriologic status at the time the culture is taken. It tells nothing as to what was there yesterday, or would have been present thereafter. It must also be granted that some of the negative cultures might have been positive if cultured in other ways, although the mediums used are the most favorable known for the growth of nonhemolytic streptococci. Several times during the past two years I have compiled statistics concerning the cultures and the percentage has remained almost constant.

These results show that infection is actually present around the root tips, tells something about the frequency of the infection, and gives an idea of the number of bacteria present. They also show that radiographic findings, while of aid, cannot be translated into terms of bacteria, nor can infection around the tip of a pulpless tooth be ruled out on a radiographic basis.

2. THE PATHOGENICITY OF THE BACTERIA FROM DENTAL FOCI

To determine the disease-producing power of the bacteria from dental foci we have injected rabbits with the original broth cultures recovered from the root tip. Two rabbits have been injected routinely with 5 c.c. each of the culture from a single tooth or with the mixed cultures from several teeth. In most instances the animals do not die as a result of the injection. If they do not, they are killed at intervals of three to six days after the injection, and the various organs examined carefully for lesions.

Other workers of course have determined the pathogenicity of mouth organisms by the injection of cultures into animals. Such a study is that of Hartzell and Henrici.[2] However, most workers have not used, for the injection, organisms grown under partial oxygen tension, or made the injections soon after isolation. These points are vitally necessary if one is to determine the true pathogenicity of the organisms. Disease-producing power in the

group dealt with here is quickly lost when these fundamental requirements are not met.

We have been able to reproduce in rabbits almost all types of lesions which can be caused by the intravenous dissemination of bacteria. The frequency with which the main groups of lesions occur is shown in Table II. The lesion most commonly found is joint involvement, the second kidney lesions, the third muscle, followed by a smaller number in endocardium, myocardium, brain, eye, and stomach and duodeum. The incidence of involvement in the animal corresponds closely to the incidence of lesions due to chronic foci, as observed clinically in man. In addition to the lesions enumerated many others have been observed such as tenosynovitis, cholecystitis, enteritis, bladder involvement, nerve lesions, and so forth.

TABLE II

LOCALIZATION OF BACTERIA ISOLATED FROM DENTAL INFECTION
(INTRAVENOUS INJECTION IN RABBITS)

NUMBER OF ANIMALS INJECTED	NUMBER OF PATIENTS	PERCENTAGE OF ANIMALS SHOWING LESIONS IN							
		JOINT	KIDNEY	MUSCLE	ENDOCARDIUM	MYOCARDIUM	BRAIN	EYE	STOMACH AND AND DUODENUM
761	255	58	34	24	18	11	6	18	13

The high incidence of lesions shows clearly the great disease-producing power of the bacteria from chronic foci and leaves no doubt that such organisms are capable of causing disease in man to a high degree.

3. EXPERIMENTAL PROOF IN INDIVIDUAL CASES OF A CAUSAL RELATION OF DENTAL INFECTION TO SYSTEMIC DISEASE

The data so far presented, however, gives in individual cases no proof of a causal relation of the dental infection to the systemic disease. Certainly the most convincing proof we have of the relation of a focus to a disease is the reproduction in animals of the condition from which the patient suffers with the organism recovered from the patient's focus. Such proof concerns the theory of Rosenow that bacteria tend to localize in certain tissues of the body due to some peculiar inherent property. The truth of the theory has been conclusively demonstrated by Rosenow and his coworkers. I have been able to present confirmatory results in diseases of the eye,[1] of the stomach,[3] of the kidney,[4] and in cases of onychia.[5] The proof seems absolute that bacteria do have such a selective tendency. Those who have questioned the theory after experimentation have failed to observe the necessary requirements as far as oxygen tension and rapidity of work is concerned. The demonstration then that animals injected with the bacteria develop lesions similar to those of the patient is the strongest evidence as to causal relation; and, likewise, evidence that in the patient and in the animal, we are dealing with the same organism. The proof that in certain cases there is this causal relation of dental infection to systemic disease is best presented by a few case histories with the protocols of animal inoculations, with the organisms recovered from chronic dental foci in the patient.

CASE REPORTS

Endocarditis and Auricular Fibrillation

CASE 1.—*History.*—L. C. H., widow, a clerk, age sixty, complained of heart trouble. She had had chorea first at age of twelve with recurrent attacks for several years. At fourteen, she had had diphtheria and at twenty-three scarlet fever. Eight years before she had scleritis. For several years she had albumin and pus in the urine. At one time removal of kidney was considered on account of the pyuria.

The patient stated she had been well up to 1912, eleven years before admission, when she had a severe attack of influenza. Two weeks later she began to have arthritis which persisted for six months. She was then well for several months, after which she began to have attacks of rapid and irregular heart. She had to give up work for seven weeks at this time on account of the heart symptoms. About once a year since this initial attack she had had an attack of heart trouble incapacitating her for work for six weeks to four months. During the past year the attacks have been occurring every few days and lasting only a few days at a time. The symptoms were worse on exertion. At times the ankles were swollen. Recently the patient had been to the Mayo Clinic where a diagnosis of paroxysmal auricular fibrillation was made.

On examination on admission, there was a definite aortic insufficiency without demonstrable cardiac enlargement. The heart rate was slow and regular except for an occasional extra systole. The blood pressure was 140/70. The urine showed a few pus cells in clumps.

Fig. 2.—Large vegetations on valves of heart of rabbit injected with the culture from infected teeth of Patient 1, who was suffering from auricular fibrillation and aortic insufficiency.

There were eleven pulpless teeth, only four of which showed definite radiographic evidence of infection.

Animal Inoculations.—The lower right second bicuspid and first and second molars were extracted first. All showed a profuse growth of nonhemolytic streptococci. Two rabbits were injected. One had at autospy a few endocardial vegetations, a few abscesses in the medulla of the kidney, and a small amount of purulent fluid in the joint. The other rabbit showed a massive vegetative endocarditis of the tricuspid valve (Fig. 2), a few lesions in the myocardium, and slight involvement of the joints. One rabbit was injected with the cultures from the lower left bicuspid and second molar. At autopsy a few vegetations on the heart valves, numerous small abscesses in the wall of the left ventricle, a purulent arthritis and a few kidney abscesses were found. Two rabbits were injected with the cultures from the remaining teeth. One was dead the following day. There were many hemorrhages in the endocardium of the left ventricle, and at the base of the papillary muscles. There were also a few hemorrhages and small vegetations in the right auricle near the ventricle.

The other rabbit died two days after injection and at autopsy showed only early vegetations on the mitral and the tricuspid valves, and mural thrombi in the right auricle.

Chronic Arthritis

CASE 2.—*History.*—R. K., A salesman, age twenty-seven, complained of rheumatism. He had never been sick until the present illness. The tonsils had been removed in 1919, four years before. The present illness had begun four weeks before admission with an eruption on the hands and trunk followed by an arthritis of the right wrist. When first seen all the larger joints and the finger joints were red, painful, and stiff. He could walk only with the aid of crutches. His eyes had been injected and painful. There was a raised pink macular eruption with clearing centers over the shoulders, arms, chest, and back, resembling erythema multiforme. The leucocytes were 13,350. The urine was negative and the Wassermann test negative.

There were only two pulpless teeth both of which showed bone absorption at the apex (Fig. 3). These were extracted. Both showed a profuse growth of green producing streptococci.

The patient had come in on crutches. He felt better immediately after the teeth were extracted. The following day all the joint symptoms had disappeared. Two days later he again came in on crutches. Examination showed that the sockets were not draining. They were opened up again. The symptoms disappeared immediately and did not return. The skin eruption also cleared up quickly.

Animal Inoculations.—Two rabbits were injected. Both at autopsy had a very marked purulent arthritis and kidney abscesses. The organism injected was recovered in pure culture from the joint and kidney lesions.

Fig. 3.—Infected teeth of Patient 2. The cultures from these teeth produced very marked arthritis in rabbits on intravenous injection.

Acute Facial Paralysis

CASE 3.—*History.*—L. M. H., a plumber, age thirty-nine, complained of facial paralysis. One year previously, he had suffered from dizzy spells and on examination was found to have albumin in the urine. The facial paralysis had appeared suddenly five days ago without any pain.

On examination the right side of the face was completely paralyzed. No other cranial nerves were involved. The blood pressure was 130/85. There was no anemia. The Wassermann test was negative. The urine examination showed a specific gravity of 1.023, and albumin four-plus, with many granular casts. The phenolsulphonephthalein excretion and the blood urea nitrogen were within normal limits. The dental radiographs showed four pulpless teeth all presenting evidence of periapical infection.

Animal Inoculations.—Three rabbits were injected with the mixed cultures from the four extracted teeth. One developed a paralysis of the hind legs forty-eight hours after injection and was killed. At autopsy all organs were negative except the brain and spinal cord which showed marked injection, some exudate at the base of the brain (Fig. 4) and hemorrhage in the caudal end of the cord. The second rabbit showed no symptoms at any time and at autopsy only some vegetations on the heart valves were found. The third rabbit, two days after injection, had a paralysis of the left ear and a pericorneal injection which gradually cleared up. When killed the only lesion found was a purulent arthritis.

Recurrent Hyalitis

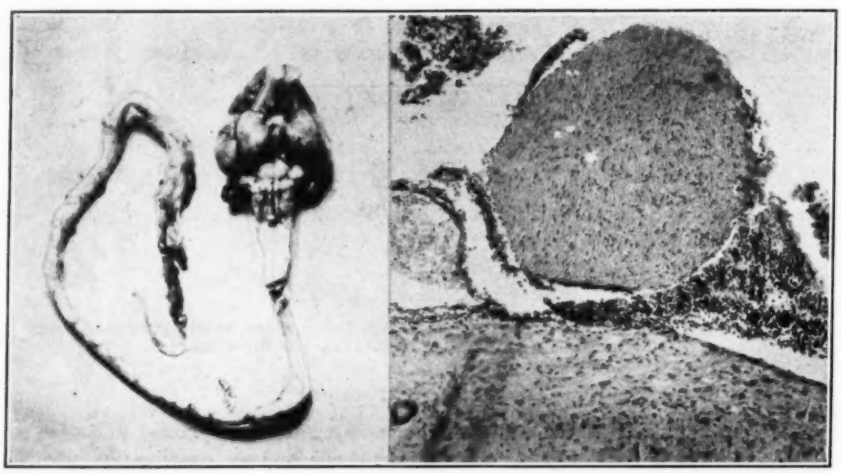
CASE 4.—*History.*—L. P., a steam fitter, age twenty-five, was first seen June 25, 1920. He complained of something flying around in front of the right eye. This was first noticed

about three weeks previously following an attack of influenza, and had become gradually worse.

The fundus was seen poorly. No hemorrhages were present. Many fine dust-like opacities and some larger ones were floating freely in the vitreous, also shreds of hyaloid tissue to which were attached numerous dust-like opacities. There was no opacity of any kind to be found in the aqueous or on the back of the cornea and no inflammation was apparent. Vision in right eye 20/100.

Under treatment the vision became better, 20/50, and at one time 20/40. Between June, 1920, and February, 1922, the patient has had four light attacks and one very severe one, which left the eye almost without a reflex. These exacerbations did not come on suddenly, as they would have if they had been recurrent hemorrhages. From the time of his first visit the patient was urged to have all his pulpless teeth extracted, but several were not removed.

A general physical examination February 24, 1922, was negative. The tonsils had been removed. Blood: red cells, 4,632,000; hemoglobin, 90 per cent; white cells, 7,400; differentia count; PMN, 55.5 per cent; PME, 4.5 per cent; PMB, 2.0 per cent; SM, 25.5 per cent; LM and Trans., 12.5 per cent. The urine showed no abnormality and the Wassermann test was negative. Radiographs of the teeth showed that the upper left cuspid (Fig. 5-*B*), and lower left second bicuspid were pulpless with poor canal filling and some bone absorption at the root tip. The lower left lateral incisor was a peg tooth, the root was poorly filled and there was a large alveolar abscess. The three pulpless teeth were extracted. The brain agar cultures showed no growth from the lower left lateral incisor, and only a few colonies from the lower left second bicuspid. The culture of the upper left cuspid showed an infinity of colonies of a nonhemolytic streptococcus. (Fig. 5-*A-C*.)



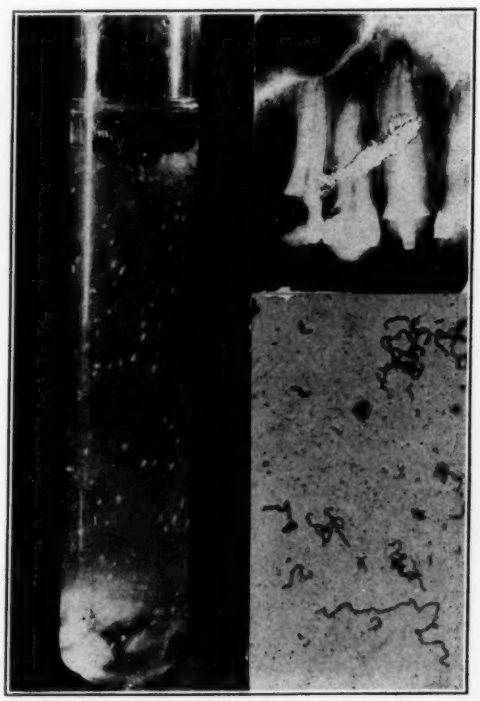
A. *B.*

Fig. 4.—*A*, brain and spinal cord from rabbit injected with the cultures from the teeth of a patient suffering from an acute facial paralysis. Note the hemorrhage and infection at the base of the brain and in the caudal end of the cord. *B*, photomicrograph of section from base of brain shown in *A*. Note the exudate around the cranial nerve.

Animal Inoculations.—March 1, 1922, two rabbits were injected with the broth culture from the upper left cuspid. One developed numerous patches of choroidoretinitis and died three weeks later. The autopsy findings were ascites and very large white kidneys. The other developed exudate in the anterior chamber and corneal opacities of both eyes twenty-four hours after inoculation. The following day the eyeballs were extremely red (Fig. 6-*A*). The right iris was discolored all the way around and the left partly around with milkish grey exudate (Fig. 6-*B*). There were greyish deposits on the cornea. The animal was killed. The autopsy was negative except for the eye findings. The streptococcus

was recovered by smear and culture from both eyes. March 5, two more rabbits were inoculated with the streptococcus recovered from the left eye of the preceding rabbit. One developed circumcorneal infection and a choroidoretinitis and died thirteen days later. Autopsy showed only kidney abscesses. The other developed a very marked injection of both eyes and died within twenty-four hours. A short chain streptococcus was recovered from the eye. Two rabbits injected with this culture died in a few hours without showing any localized lesions. Two rabbits injected with the culture from the right eye of one of the second set of rabbits, developed patches of choroidoretinitis and were killed six weeks later. One showed no lesions, the other only a purulent arthritis.

Three weeks after the original apical cultures had been made, organisms were removed from the agar tube (Fig. 5-*A*) with a sterile pipette, grown in broth for twenty-four hours, and injected into three rabbits. One developed a cloudy vitreous and died four days after



B.

C.

A.

Fig. 5.—*A*, original culture tube from tooth shown in *B*. *C*, photomicrograph of streptococcus culture tube at left.

inoculation. Autopsy revealed an arthritis. The second animal showed a pericorneal injection three days after inoculation. The iritis gradually cleared. The vitreous of the right eye became increasingly hazy. The red reflex was lost entirely and there was little pupillary light reflex (Figs. 6-*C* and 7-*B*). The animal died eight days after inoculation. Necropsy was negative except for the findings. The patient's vision at the present time is 15/100.

Acute Pyelonephritis

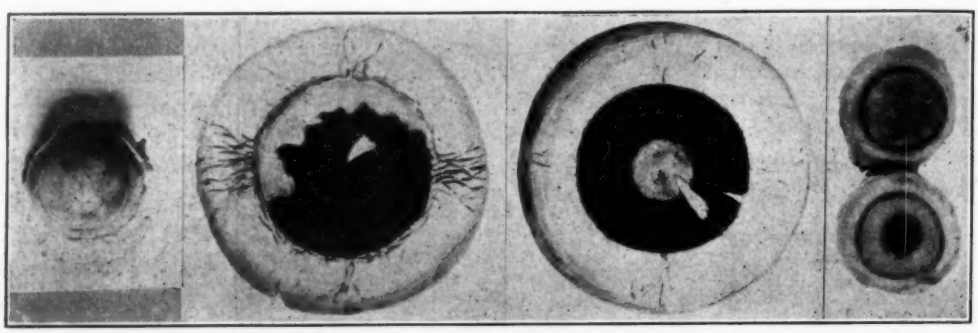
CASE 5.—*History.*—W. W., a medical student, age thirty-four, stated that two weeks previously he had suffered from frequent burning urination, hematuria, chills, and fever as high as 102 degrees. The symptoms improved at first under medication but the chills and fever recurred. He had had a similar attack nine years previously. There had been no other illness. The tonsils were cleanly removed. Physical examination was negative. The urine showed gross blood, many pus cells and a short chain streptococcus which was

recovered by culture. One bicuspid tooth (Fig. 8-*A*) held a large inlay under which the pulp had died. There was no area of rarefication at the tip. The tooth was extracted. From the tip a pure culture of a green producing streptococcus (str. fecalis) was obtained.

Animal Inoculations.—Two rabbits were injected with the culture of the streptococcus. One showed at autopsy multiple abscesses in the pyramids and a purulent arthritis (Fig. 8-*B*). The other showed an acute hemorrhagic nephritis and a few endocardial vegetations. The patient's urine returned to normal and there have been no further symptoms.

Duodenal Ulcer

CASE 6.—*History.*—H. A. A., a business man, age forty-four, had several gastric hemorrhages in December, 1922. For six months previous to this he had had indigestion,



A. *B.* *C.* *D.*

Fig. 6.—*A*, photograph of eye of rabbit twenty-four hours after injection of streptococcus shown in Fig. 5-*C*. *B*, drawing of eye of rabbit injected with same culture. *C*, eye of rabbit several days after injection with attenuated culture from same patient. Note the loss of light reflex due to involvement of vitreous humor. *D*, eyes of rabbit injected with culture from dental foci of same patient one year after the first cultures were made. The eye below is normal, the one above has the anterior chamber filled with purulent exudate.

A. *B.*

Fig. 7.—*A*, photomicrograph of eye shown in Fig. 6-*B*. Note the cellular infiltration of choroid. *B*, photomicrograph through vitreous humor of eye shown in Fig. 6-*C*. Note the cellular infiltration in vitreous humor.

consisting principally of a feeling of fullness after eating. A diagnosis of duodenal ulcer was made. Several teeth were extracted at this time. There were no further hemorrhages and the symptoms were largely relieved although the patient states that he still has indigestion at times for which he takes soda.

Radiographs in June, 1924, showed one tooth of questionable vitality and one pulpless tooth with little radiographic evidence of infection. At the site of extraction of the upper left bicuspid and first molar some filling material was remaining, and the surrounding bone showed evidence of infection (Fig. 9-*C*). The two teeth were extracted and the infected

bone curetted. Cultures in deep tubes of glucose brain broth agar showed a short chain streptococcus in all.

Animal Inoculations.—Two rabbits were inoculated with the mixed broth cultures. One rabbit was dead the following morning and showed many hemorrhages in the duodenum. The second rabbit was killed. This one showed also many hemorrhages in the first third of the duodenum without lesions elsewhere (Fig. 9-*A*). In order to determine whether the area of infected bone might play a part in the causation of the ulcer one rabbit was injected with 5 c.c. of the broth culture from this area only. At autopsy twenty-four hours later the duodenum showed massive hemorrhages (Fig. 9-*B*). There were no other lesions.

Multiple Onychia

CASE 7.—*History.*—M. K., a housewife, age fifty-one, was first seen in relation to her present infection on January 10, 1922. She gave no history of the acute infectious diseases of childhood. Until three years before she had led an exceedingly active life on a farm. She had had palpitation of the heart and soreness in the chest for many years. At inter-

A. *B.* *C.*

Fig. 8.—*A*, tooth of patient suffering from an acute pyelonephritis. The pulp had died under the inlay. There was no radiographic evidence of infection. *B*, kidney of rabbit injected with the streptococcus recovered from the tooth shown in *A*. Note the abscesses in the medulla. *C*, photomicrograph through medulla showing an abscess.

vals during the last twenty-three years she had had painful swellings of the larger joints. She had had nycturia two or three times without pain on voiding.

Twelve years before, she began to have trouble with her finger nails. This consisted of swelling, redness and tenderness around the nail roots. At times pus could be expressed. The nails of all the fingers were successively involved, as well as the nails of the great toe on the left foot. The infection usually ended with exfoliation of the nail. There was no history of injury or of the use of irritating substances. The trouble continued until five years before when the patient was referred for dental treatment. Four teeth were found to have periapical abscesses and were extracted. One tooth although pulpless, was negative in the radiograph and was filled and allowed to remain. Following the dental treatment, all the active nail lesions cleared up, as did the arthritis.

On January 10, 1922, the patient returned because she had begun to have pain and redness around one finger nail. She was also having palpitation of the heart. She now had no joint symptoms.

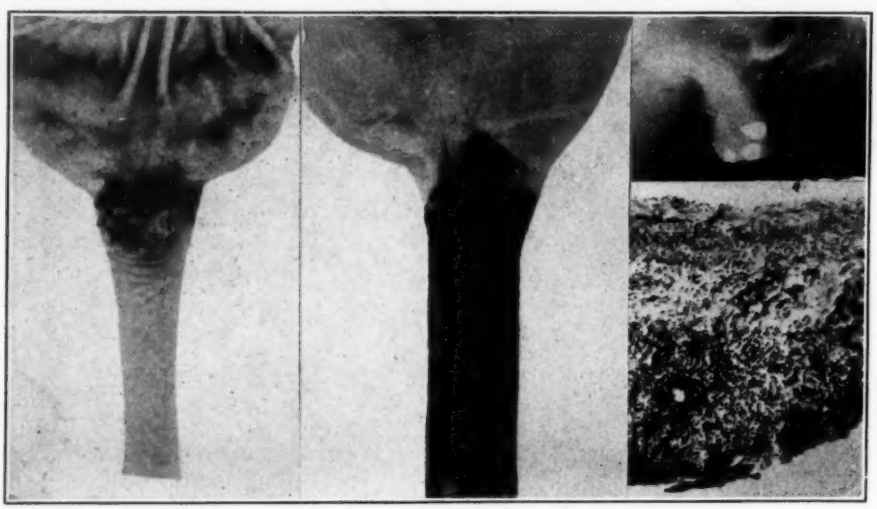
On examination the middle finger of the left hand showed marked swelling and redness around the nail root. The nail was tender on pressure. No pus could be expressed. There was no glandular enlargement. The right tonsil was red and showed a few plugged crypts. The heart action was rapid; the sounds were distinct with a tendency to gallop rhythm; the blood pressure was; systolic, 110; diastolic, 65.

A blood count showed 5,592,000 red cells, 4,950 white corpuscles, and 90 per cent, hemoglobin (normal standard). A differential count of 500 cells showed; polymorphonuclear neutrophiles, 58 per cent; eosinophiles, 1 per cent; basophiles, 0; large mononuclears, 7 per cent; and lymphocytes, 34 per cent. The Wassermann test was negative.

A radiograph of the teeth showed areas of rarefaction around the roots of the lower right molar. All other teeth were vital. There was no pyorrhea.

The lower right second molar was extracted. The nail infection quickly subsided. A urine examination six weeks later showed no albumin or pus cells. The palpitation of the heart disappeared. The patient was seen several months later. There had been no return of the nail infection.

Animal Inoculations.—A culture on blood agar of the roots of the extracted tooth showed a profuse growth of Streptococcus nonhemolyticus 1 (Holman). On January 13,



A. *B.* *C.* *D.*

Fig. 9.—*A*, lesions in duodenal bulb produced by the intravenous inoculation of bacteria recovered from areas of dental infection of Patient 6, who was suffering from a duodenal ulcer. *B*, duodenum of rabbit following the intravenous injection of streptococcus recovered from the infected bone (*C*) from same patient. *C*, infected bone at site of extraction of teeth two years previously. Note the small pieces of filling material remaining. *D*, photomicrograph through lesion in duodenum.

one rabbit was injected intravenously with a broth cutlure of the organism. Four days later a second injection was given. Eight days after the first injection the animal was killed. The postmortem examination showed hemorrhages in the lumbar muscles, a few small abscesses in the cortex of the kidneys and purulent fluid in the shoulder joints. The organism was recovered in pure culture from the joint fluid. Around the nail root of the second toe of the left fore foot there was marked swelling and injection (Fig. 10-*A*). There was hemorrhage around the nail roots of the other toes of the same foot. The other toes were normal. A drawing of the section of the toe which revealed grossly the most marked change shown in Fig. 10-*B*. The nail was removed before the section was cut. The area of polymorphonuclear leucocyte infiltration is evident (Fig. 10-*C*).

The organism recovered from the joint of the first rabbit was injected into two others. These showed muscle hemorrhage and a purulent arthritis and abscesses of the kidney but

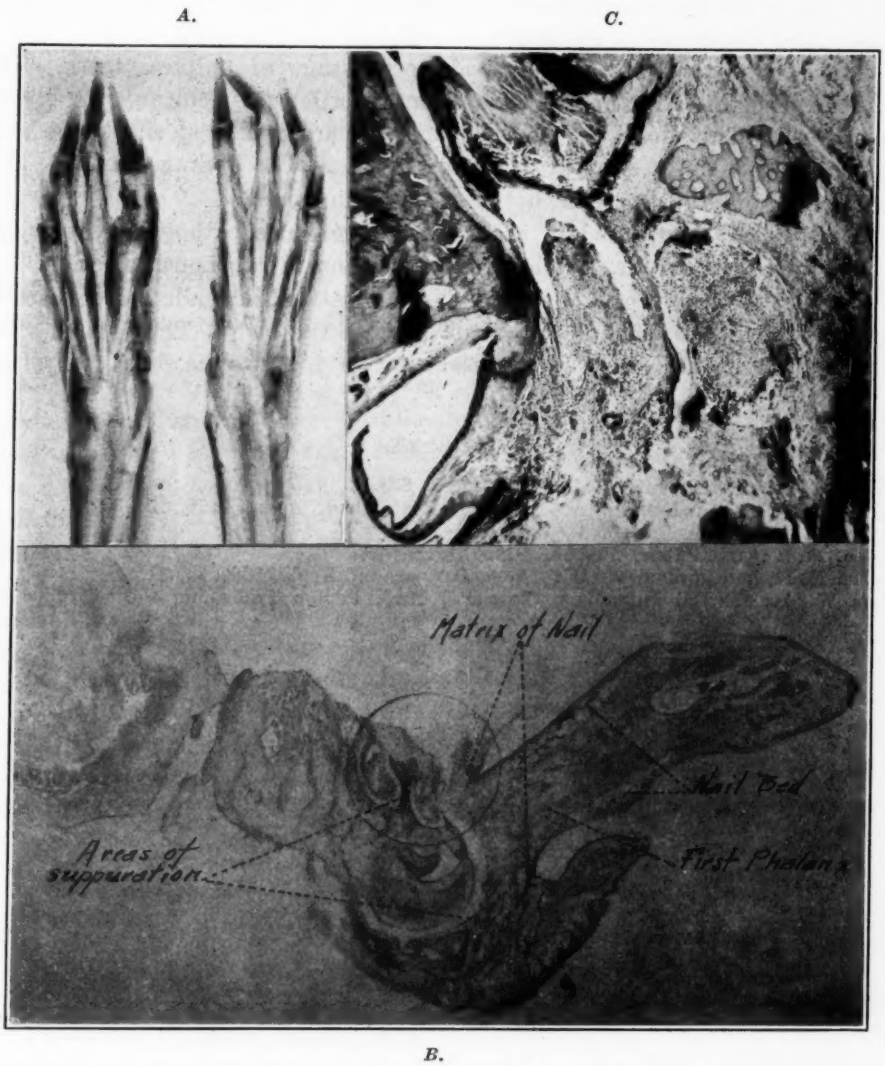


Fig. 10.—*A*, toe of rabbit following the injection of streptococcus from patient who was suffering from multiple onychia. *B*, drawing of section of toe after the nail was removed. *C*, photomicrograph of area at base of nail. Note the areas of polymorphonuclear infiltration.

no involvement of the nails. The organism was carried through two more sets of rabbits with similar results.

SUMMARY AND CONCLUSIONS

I should like to summarize the points which I have attempted to emphasize in this paper as follows:

A very high percentage of teeth, which are negative in the radiograph, harbor infection. The radiograph should never be depended on to eliminate a tooth as a possible focus of systemic disease.

A fairly large percentage of teeth which were positive in the radiograph did not harbor any infection or sufficient infection to be a factor in systemic disease at the time the culture was taken. In such cases, the in-

fection has probably run its course and become bacteria free, just as happens in infections elsewhere in the body.

The periapical tissues of a certain percentage of pulpless teeth, either positive or negative in the radiograph, are sterile when cultured in glucose brain broth. This does not prove that such are really sterile, since some other method of culture might reveal organisms. The findings suggest, however, that a pulpless tooth is not necessarily infected.

The bacteria concerned in chronic foci are quite pathogenic, as judged by their ability to produce lesions in animals on intravenous injection.

In selected cases, one can prove an unmistakable tendency of bacteria from chronic foci to localize in certain parts of the body. These cases afford valuable experimental proof of a causal relation of a chronic focus to systemic disease.

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THE PARATHYROID GLANDS AND THEIR IODINE CONTENT*

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PERIODICALLY during the past twenty-five years, articles have appeared in the literature relative to the presence of iodine and its significance in the parathyroid glands. The bulk of the evidence at hand is clean cut, and yet confusion exists, due in part, at least, to an incomplete knowledge of the comparative anatomy of these glands in different species, and to a failure in discriminating between the internal and external glands. To further complicate matters, there appears under parathyroid preparations in *New and Nonofficial Remedies*, a description of the desiccated gland, which includes the iodine content. It is true that these bodies may contain a trace of iodine, but the fact as thus conveyed naturally may lead those unacquainted with the collection of material for the manufacture of parathyroid preparations to conclude that it is contaminated with thyroid tissue. Under the above-mentioned description it is definitely stated that the product represents the external bovine glands. In this species the external glands are distinct, and separated by a considerable margin from the thyroid glands. Contamination by the thyroid in this preparation is, therefore, improbable. At this time it seems pertinent to call to mind the facts revealed by past publications and the observations of the present writers.

IODINE CONTENT OF THE THYROIDS AND PARATHYROIDS

Baumann,[1] in 1896, by adapting a colorimetric method of analysis originally devised by Rabourdin,[2] discovered that the thyroid glands contained a considerable quantity of iodine. This method consisted essentially in the ashing of desiccated gland substance in an alkaline potassium nitrate medium, with subsequent acidification and extraction with chloroform, which yields a violet color, and comparing the color with that developed by a known amount of iodine in a similar mixture.

Gley[3] (1897), by means of this method, reported that the parathyroid glands of rabbits contained more iodine than the thyroids. He used this information in evolving the theory that the parathyroids were an embryonic form of thyroid tissue. Gley's findings were not corroborated by other observers for Chenu and Morel[4] (1904) found but very slight traces of iodine in the external parathyroids of dogs, rabbits, and chicks; and Estes and Cecil[5] (1907) could recover no iodine from the parathyroids of the cow, sheep, and man, and from none to infinitesimal amounts in the dog, and none to 0.06 mg. in three grams of desiccated glands of the horse. As the same method of analysis was used in all of these reports, it is possible that some of

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the differences can be explained by the assumption that Gley either had considerable thyroid contamination in his parathyroid preparations, or his technic was faulty. Suffice it to say that though the parathyroid glands may contain traces of iodine, it is of little consequence.

Baumann[6] (1896) noted an absence of iodine in the thyroids of a great majority of young infants. This observation was corroborated by Miwa and Stöltzner[7] the following year, and, in 1900, by Mendel,[8] who in using Oswald's[9] modification of Baumann's method, could find no iodine in the thyroid glands of four infants at full term, but recovered 0.07 mg. from the glands of two infants twenty-four days of age.

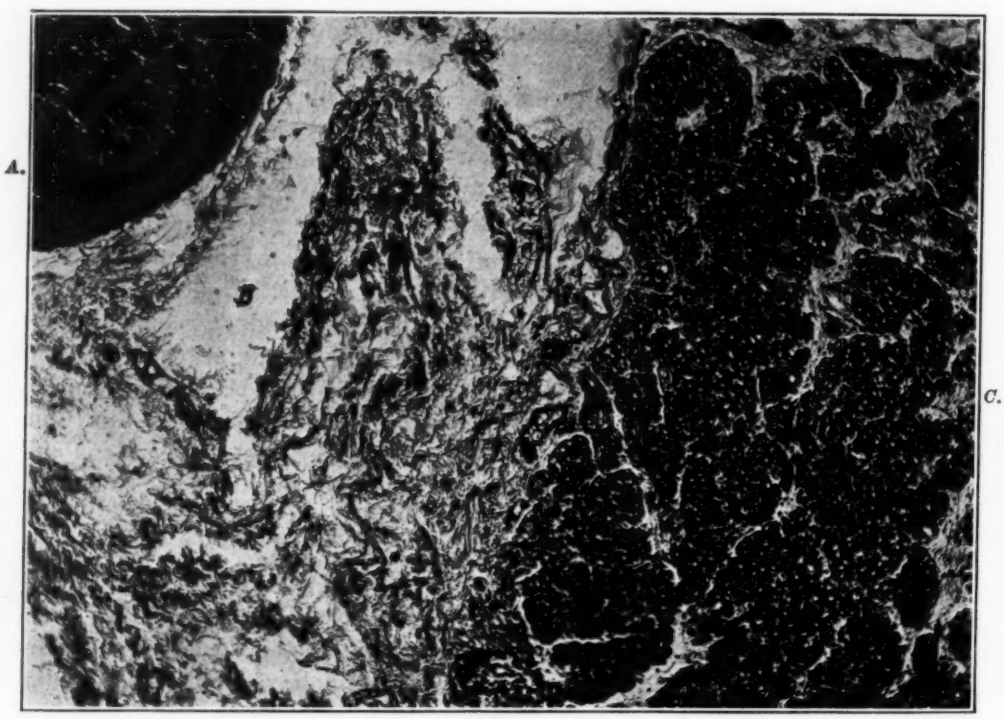

Fig. 1.—Parathyroid and lymph glands. Low power. 16 mm. objective, 4X ocular, 24″ bellows length. *A*, Lymph gland; *B*, connective tissue; *C*, parathyroid gland.

THE IODINE CONTENT OF OTHER GLANDS

Baumann[1] could find no iodine in the hypophysis, but recovered a slight trace in the calf's thymus on analysis of a large quantity of material (200 gm. of desiccated gland). Schnitzler[10] (1896) reported that he had found iodine in the human hypophysis. In 1898, Cunningham[11] stated that he could not confirm Baumann's observations on the calf's thymus, for on repeated analyses, he was uniformly unsuccessful in recovering iodine. Mendel[8] reported the absence of iodine in the thymus of infants from birth to twenty months of age. In the same article, he states that an analysis of the thyroid and retrosternal accessory thyroid of a human being resulted in the finding of more iodine in the latter than in the former. Wells,[12] (1909) in an analysis of the pituitary glands of twenty-five human beings, twenty-two of whom had

not received iodine therapy antemortem, and three of whom had received such treatment, found no iodine in the former, but a distinct trace in the latter group. Denis[13] (1911) using the Baumann-Riggs method[14] (essentially the Baumann method wherein carbotetrachloride is substituted for chloroform), found no trace of iodine either in the pituitary glands of sixteen humans who had received no iodine therapy antemortem, or in the glands of five who had. Seaman[15] (1920) by the latter method was unsuccessful in recovering iodine from thirty, fifty, and one hundred gram quantities of desiccated sheep's pituitary glands.

From the foregoing, it is seen that traces of iodine have been found by

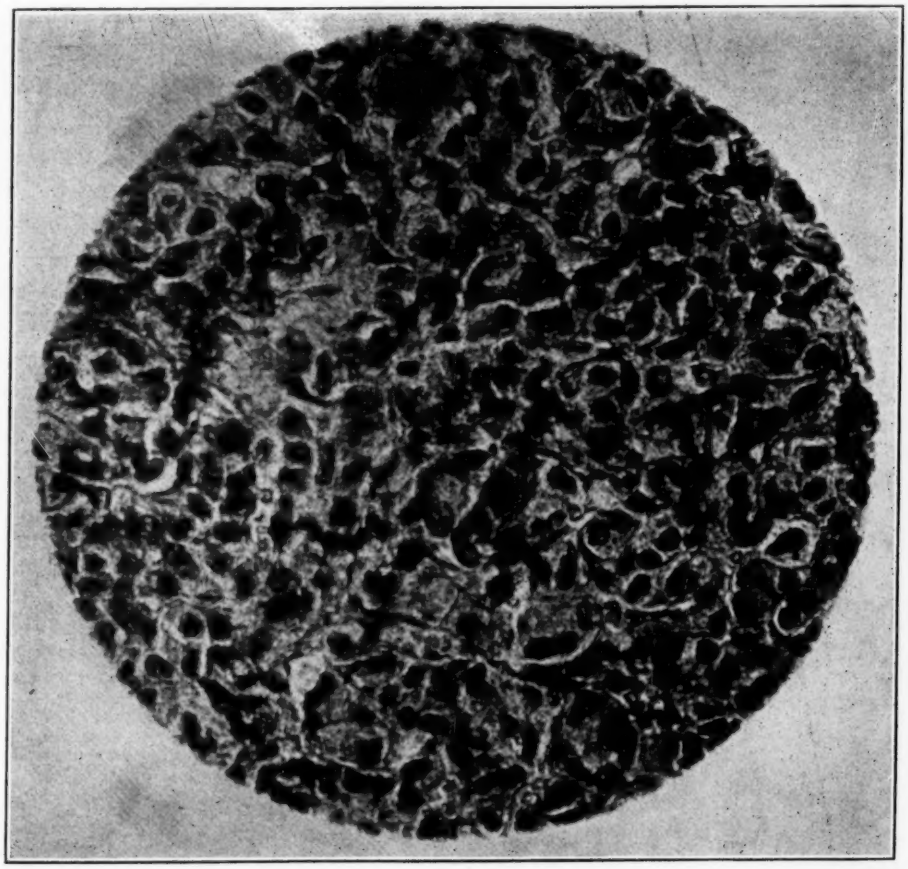

Fig. 2.—Parathyroid gland. High power. 4 mm. objective, 4X ocular, 24" bellows length.

some observers and not by others in glands other than the thyroids and parathyroids. It may well be possible that this discrepancy in results is due either to variation in quantities used for analysis, or to the limitations of the methods of determination, for they are all essentially the same,—the Baumann or its modification. To correlate this literature, we have analyzed several different gland tissues simultaneously by another method. In addition, we have studied fresh parathyroid glands histologically for the presence and absence of thyroid inclusions both within the gland substance and adherent to its capsule.

DOES THE EXTERNAL BOVINE PARATHYROID CONTAIN THYROID TISSUE?

The parathyroid material examined consisted of 43 external bovine glands collected from freshly slaughtered steers, and 109 such glands as collected commercially and frozen for preservation. No difficulty was encountered in collecting the glands, nor in distinguishing them from the neighboring lymph glands in this species. Macroscopically, the glands varied in color from a light tan to dark brown, but in all a yellowish tinge prevailed; they were firmer in consistency and more opaque than the lymph glands; they were

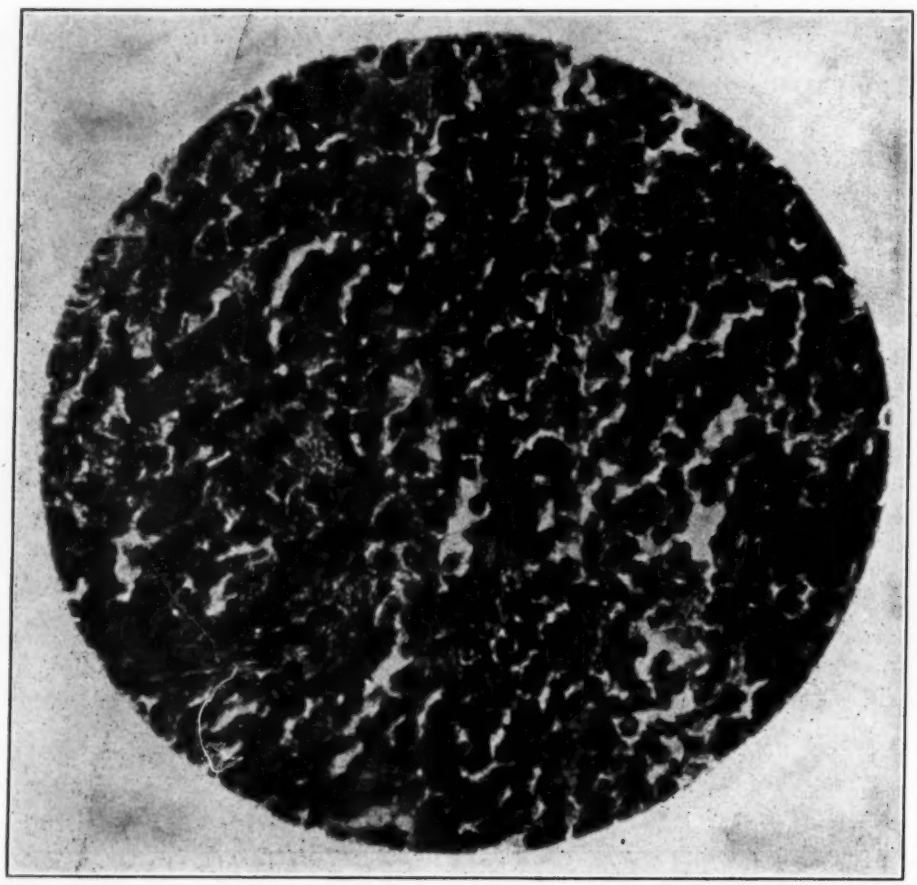

Fig. 3.—Lymph gland. High power. 4 mm. objective, 4X ocular, 24″ bellows length.

easily identified by examination of a cut surface, if some doubt was entertained by external appearances. Microscopically, the parathyroid glands showed a distinct structure very different from that possessed by the lymph glands, as shown in Figs. 1, 2 and 3.

From four to eight sections were made at several levels of each gland examined. In none was found thyroid tissue, either intraglandularly or adherent to the capsule. The glandular structure varied somewhat in the different specimens, but only in the relative amount of glandular, as compared

with connective tissue. In none of the glands examined was there found the colloid substance described by Schaper.[17] The glands we collected contained more lymphoid tissue adherent to the capsule than did the commercial product. These observations serve to show that there is no danger of contaminating parathyroid preparations with thyroid tissue at the time of collection from the animal.

THE IODINE CONTENT OF VARIOUS GLANDS

Specimens of desiccated parathyroid, pituitary, thymus, and ovary were chosen for analysis. The first two were obtained from the ox and were desiccated with acetone, the last two were obtained from the calf and hog respectively and desiccated without the assistance of acetone.

The Hunter[16] method of iodine determination has been used by one of us for some years in estimating the iodine content of desiccated thyroid preparations and found to be very reliable and accurate. This method consists essentially of ashing in an alkaline fusion mixture (sodium carbonate, potassium carbonate, and potassium nitrate), with subsequent oxidation of the resulting iodides into iodates, and estimation of the iodine by iodimetry. Since, in our work the amount of iodine was expected to be small, greater care in the selection of reagents, and in the titration was necessary. All reagents were of the highest purity obtainable and were tested for their iodine content before using. Iodine was absent in all, as determined by the silver precipitation test with subsequent solution of the silver chloride with ammonia. A special one cubic centimeter burette graduated into hundredths of a cubic centimeter was used for the titrations. Blank determinations of the reagents used were made after repeating all performances necessary in the preparation of the gland substances for the iodimetric analyses. The blank titrated constantly at 0.1 c.c. of N/200 sodium thiosulphate.

The results of analysis of the various glands above mentioned are included in Table I.

TABLE I

GLAND	WEIGHT DESICCATED GLAND IN GM.	$Na_2S_2O_3$* C.C. N/200	IODINE MG.	IODINE %
Parathyroid	2	0.15	0.09511	0.00476
Parathyroid	2	0.10	0.06341	0.00317
Parathyroid	2	0.20	0.12682	0.00634
Ovary	2	0.10	0.06341	0.00317
Pituitary	2	0.15	0.09511	0.00476
Thymus	2	0.10	0.06341	0.00317

* c.c. N/200 $Na_2S_2O_3$ represents the amount after deduction of 0.1 c.c. for the blank.

The accuracy of this method when used in the estimation of as minute amounts of iodine as is contained in the body tissues, other than the thyroid, is limited. The end point in the titration cannot be determined within 0.05 c.c. However, all of the analyses were in such close proximity to each other as to warrant the conclusion that but a very slight trace of iodine is contained in the glands examined and that the iodine is quite uniformly dis-

tributed throughout the animal glandular system, the thyroid excepted. These findings are essentially a corroboration of the majority of past observations.

CONCLUSIONS

1. Parathyroid preparations made from the external bovine glands were not contaminated with thyroid tissue.

2. The iodine content of the parathyroids is of no greater magnitude than that of the ovaries, pituitary and thymus.

3. The iodine found in these glands probably represents the normal distribution of that substance throughout the body tissues.

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NARCOTIC AND TOXIC POTENCY OF ALIPHATIC ALCOHOLS UPON RABBITS*

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INTRODUCTION

THE purpose of the investigation here reported was to determine the toxicity of the lower aliphatic monatomic alcohols when administered by mouth. Such data were not available for some of the alcohols studied. Moreover, the propriety of comparing the results of different investigators studying different alcohols by different methods is at times questionable. Some of the other criteria for comparing and studying the alcohols pharmacologically are purely arbitrary and are, therefore, not directly indicative of oral toxicity ratios. Intravenous administration may cause emboli. Neither the adequacy nor the necessity of hirudin in such injections has been shown. In subcutaneous administration, absorption is usually poor or accompanied by necrosis of a great deal of tissue. Intraperitoneal administration† is impracticable because solutions for injection containing comparable doses of the different alcohols either cannot be prepared on account of their insolubility in suitable media or, if prepared, are too bulky.

REVIEW OF LITERATURE

The earliest investigation of the narcotic and toxic effects of a series of homologous alcohols reported, is that by B. W. Richardson.[46] In studying the relation between molecular weight and physiologic action he deduced the so-called "Richardson Law," "Weight, *caeteris paribus,* intensifies action and makes it more prolonged." The following year, Rabuteau[44] concluded from his experiments that "the alcohols of the series $C_nH_{2n}+{}_2O$ are the more active as the group CH_2 enters a larger number of times into their structure." Since the time of these two investigators, numerous experiments in which various forms of intact plant[21, 38, 50, 51] and animal[3, 4, 9, 18, 25, 42, 57] life, surviving organs,[22, 30, 59] nerves,[8, 17, 24, 45, 55] muscles,[5] enzymes,[36, 56] and even physicochemical experiments[28, 49] were employed, have revealed no more important or acceptable quantitative pharmacologic generalization. The mechanism of action, both on the unicellular and multicellular organisms, however, is better understood qualitatively. Mathematical discussions of the toxicity of alcohols are still a matter of dispute and the mechanism of pharmacologic action is still a fruitful field for investigation.††

*An abstract of the results of this investigation was presented at the New Haven meeting of the American Society for Pharmacology and Experimental Therapeutics, December 29, 1921.

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†Unpublished experiments by the authors.

††A thorough discussion of the results obtained by a number of investigators is given by Winterstein[58] in his monograph on narcosis.

TABLE I

MOLAR NARCOTIC POTENCY OF ALIPHATIC ALCOHOLS

INVESTIGATOR	METHOD OF STUDY	TEST ANIMAL OR SUBSTANCE	MEOH	ETOH	PROH	ISO-PROH	N-BUOH	ISO-BUOH	SEC-BUOH	TERT-BUOH	N-AMOH	ISO-* AMOH	SEC-AMOH	TERT-AMOH
(2)	Oral	Rabbits	0.55	1.00	2.25			4.7				6.8		
			0.1763	0.0967	0.0430			0.0205				0.0142		
(6)	"	"		1.00	1.30									
				0.0326	0.0250									
(42)	Immersion	Tadpoles	0.51	1.00	2.6	2.2	7.6	6.4		2.2		12.6		5.10
			0.57	0.29	0.11	0.13	0.038	0.045		0.13		0.023		0.057
(38)	"	Daphnia	0.50	1.00	3.0			5.0				15.0		
			1.2	0.6	0.2			0.12				0.04		
(20)	"	Phoxinus (Ellritzen)	0.42	1.00	3.75	1.70	14.2	10.30			55.0	40.0		
			0.594	0.250	0.0666	0.1496	0.0176	0.0243			0.0045	0.0062		
(35)	"	Arenicola larvae	0.41	1.00	3.1		11.80					44.8		
			0.25	0.103	0.0337		0.0087					0.0023		
(41)	"	Young trout	0.35	1.00	2.2			8.5				27.6		
			0.859	0.298	0.142			0.0351				0.0108		
"	"	2 yr. old trout	0.34	1.00	3.2			11.0				45.2		
			0.828	0.282	0.09			0.0257				0.0062		
(25)	"	Allolobophora (worm)	0.50	1.00			16.7				50.0	33.3		12.5
			2.00	1.00			0.06				0.02	0.03		0.08
(22)	Perfusion	Frog heart	0.32	1.00	3.30	1.80	11.6	8.9		1.9		30.9		6.5
			3.74	1.206	0.369	0.655	0.109	0.135		0.637		0.39		0.184
(83)	Immersion	Tadpoles		1.0	2.8		8.3							
				1.5	0.54		0.18							
(4)	"	Paramecia	0.45	1.00	4.7	2.7	10.4	13.0						
			1.2475	0.567	0.121	0.213	0.055	0.0437						
Range of molar values:			0.32–0.55	1.00	1.30–4.7	1.7–2.7	7.6–14.2	4.7–13.0		1.9–2.2	50.0–55.0	6.8–45.2		5.1–12.5

The lower figure of each set is the absolute molar value of the alcohol in question, required to produce the designated result; the upper figure indicates the relative molar potency when the absolute value for ethyl alcohol is taken as a unit.

*Many investigators report results on amyl alcohol; unless further identified by chemical constants, the assumption has been made that they were really using isoamyl alcohol.

TABLE II

MOLAR TOXICITY OF ALIPHATIC ALCOHOLS

INVESTIGATOR	METHOD OF STUDY	TEST ANIMAL OR SUBSTANCE	MEOH	ETOH	PROH	ISO-PROH	N-BUOH	ISO-BUOH	SEC-BUOH	TERT-BUOH	N-AMOH	ISO-* AMOH	SEC-AMOH	TERT-AMOH	REMARKS
(16)	Subcutaneous	Dogs	0.77	1.00	2.7	2.7		6.9				9.7	9.7		Death in 24-36 hr.
			0.2185	0.1683	0.0632	0.0624		0.0244				0.0173	0.0173		
(11)	"	"	0.81	1.00	2.8		6.1	5.6				6.2			
			0.2340	0.1890	0.0666		0.0310	0.0337				0.0306			
(2)	Oral	Rabbits	0.58	1.00	2.8			4.9				7.3			Death in approx. 24 hr. usually produced.
			0.2810	0.1616	0.0576			0.0329				0.0221			
(33)	"	"	0.54	1.00											Very small rabbits.
			0.3500	0.1880											
(6)	"	"		1.00	1.3										
				0.1085	0.0832										
(27)	Intravenous plus hirudin	"	0.52	1.00	2.5			7.2				19.8			MeOH & EtOH true toxicity; others experimental toxicity.
			0.2720	0.1416	0.0566			0.0196				0.00715			
"	"	Dogs	0.66	1.00											
			0.2270	0.1503											
(34)	"	Rabbits	0.46	1.00	6.5			16.1				38.2			Kill at once (?)
			0.4680	0.2170	0.0333			0.0135				0.00568			
(11)	Intravenous no hirudin	Dogs													
			0.1343	.....	0.0383		0.0067	0.0205				0.0125			
(39)	"	Cats	0.58	1.00	3.20	2.6	26.2	8.7			62.2	35.9			Kill at once (?)
			0.1473	0.0858	0.0270	0.0333	0.0033	0.0098			0.0014	0.0024			
(14)	"	Rabbits	0.35	1.00								33.7			
			0.5000	0.1737								0.0051			
(11)	Intraperitoneal	Dogs	0.43	1.00	2.5		3.3	3.1				9.9			
			0.1560	0.0673	0.0266		0.0205	0.0216				0.0068			
(44)	Immersion (Mol./Liter)	Frogs		1.00				3.5				12.7			Ratio-basis EtOH = 1.0.
				1.00				3.5				12.75			
(43)	"	Goldfish	0.48	1.00	2.5			5 5				19.6			Rapid death.
			0.937	0.4450	0.1750			0.0810				0.0227			
"	"	"		1.00	2 6			12.9				76.6			Death after approx. 1 hr.
				0.1303	0.0500			0.0101				0.0017			

TABLE II—CONT'D

MOLAR TOXICITY OF ALIPHATIC ALCOHOLS

INVESTIGATOR	METHOD OF STUDY	TEST ANIMAL OR SUBSTANCE	MEOH	ETOH	PROH	ISO-PROH	N-BUOH	ISO-BUOH	SEC-BUOH	TERT-BUOH	N-AMOH	ISO-* AMOH	SEC-AMOH	TERT-AMOH	REMARKS
(7)	"	Fish	0.69	1.00	1.3	1.3	31.80			31.8		70.2			"Strength" considered volume concentration
			0.5000	0.3430	0.2660	0.2660	0.0108			0.0108		0.0045			
(10)	"	Av. on 6 types fish	0.77	1.00	4.6			13.2				35.0			Death within 24 hr.
			0.2830	0.2170	0.0472			0.0164				0.0062			
(3)	"	Trout		1.00	3.2			7.8							
				0.7720	0.2430			0.0984							
(34)	Immersion	Gudgeon	0.55	1.00	2.0			3.1				5.0			Kill at once (?)
			1.25	0.6870	0.3370			0.2190				0.1380			
(9)	"	Fundulus heteroclitus	0.83	1.00				20.8				50.0			Concentration to stop gill arches in approx. 15 minutes.
			1.50	1.25				0.06				0.025			
(9)	"	Starfish eggs	0.53	1.00				8.0				25.0			Concentration to cause liquefaction.
			3.75	2.0				0.25				0.08			
(18)	"	Sea urchin eggs (Psammechinus miliaris)	0.33	1.00	4.0										Checks multiplication & division of eggs of Psammechinus miliaris.
			0.94	0.31	0.078										
(19)	"	Strongyocentrotus lividus	0.57	1.00	3.0		9.0					20.0			
			0.719	0.408	0.136		0.0454					0.0204			
(21)	"	Convoluta	0 32	1.00	3.4		11.2								
			0.938	0.304	0.090		0.027								
(4)	"	Paramecia	0.46	1.00	2.8	2.0	7.4	5.8							
			1.225	0.567	0.204	0.280	0.0765	0.0984							
(13)	Perfusion	Frog heart	0.75	1.00	1.5		4.5					26.3			To stop heart.
			1.0	1.33	2.0		6.0					35.			Ratios: MeOH = 1.0.
(31)	Perfusion	Rabbit heart	1.00	1.00	5.0		7.5					75.0			To stop heart—Ratios MeOH = 1.0.
			1.0	1.0	5.0		7.5					75.0			
(32)	"	Rabbit intestine	0.80	1.00	9.8		36.0					108.0			To inhibit contraction.
			1.0	1.25	12.25		45.0					135.0			Ratio:—EtOH = 1.
(47)	"	Frog heart	0.56	1.00	1.9	2.6		6.7		2.8		17.1			
			0.56	1.00	1.92	2.56		6.68		2.82		17.1			

TABLE II—CONT'D

INVESTIGATOR	METHOD OF STUDY	TEST ANIMAL OR SUBSTANCE	MEOH	ETOH	PROH	ISO-PROH	N-BUOH	ISO-BUOH	SEC-BUOH	TERT-BUOH	N-AMOH	ISO-* AMOH	SEC-AMOH	TERT-AMOH	REMARKS
(54)	"	Tortoise heart	**0.50**	**1.00**	**2.8**	**2.2**	**13.1**	**11.2**	**8.5**	**3.7**		**31.6**		**7.41**	
			0.106	0.0530	0.0188	0.0235	0.0040	0.0047	0.0062	0.0144		0.0017		0.0071	
(55)	Immersion	Various muscles	**0.50**	**1.00**											Ratio: MeOH = 1.0.
			1.0	2.0											
(8)	"	Motor nerves													
			1.0	1.0	10.0							20.0			
(23)	Hemolysis	Ox R B C	**0.44**	**1.00**	**3.0**		**10.2**					**35.6**			Mol. per liter to hemolyze in 5 min.
			7.34	3.24	1.08		0.318					0.091			
(49)	"	"	**0.48**	**1.00**	**2.9**							**23.4**		**6.1**	"
			8.6	4.10	1.40							0.175		0.67	
(51)	Plasmolysis	Red onion cell (April)	**0.48**	**1.00**		**1.7**									Mol. per liter which just permit plasmolysis.
			10.0	4.84		2.80									
		(August)	**0.47**	**1.00**	**2.8**	**2.0**									
			9.12	4.27	1.50	2.13									
(52)	Hemolysis	R B C ox	**1.00+**	**1.00**		**2.8**		**5.6**				**15.3**			No hemolysis in 3 hrs. (as ratios).
		dog	100.+	100.		35.67		17.89				6.54			
		man													
		rabbit													
(53)	"	R B C ox		**1.00**		**2.1**		**3.4**				**8.0**			"
				100.		47.00		29.0				12.5			
Range of molar values:			**0 33–**	**1.00**	**1.30–**	**1.3–**	**6.9–**	**3.1–**	**8.5**	**2.8**	**35 6–**	**5.0–**		**6.1–**	
			1.00		**9.8**	**2.8**	**36.0**	**20.8**		**31.8**	**62 2**	**108.0**		**7.4**	

The lower figure of each set is the absolute molar value of the alcohol in question, required to produce the designated result; the upper figure indicates the relative molar potency when the absolute value for ethyl alcohol is taken as a unit.

*Many investigators report results on amyl alcohol; unless further identified by chemical constants, the assumption has been made that they were really using isoamyl alcohol.

A résumé of the literature on the comparative narcotic and toxic action of various alcohols found in the course of this investigation is given in Tables I and II. The data are presented on the molar basis (gram-molecules per liter of solution), the customary method of chemical comparison. They may be readily converted into grams or cubic centimeters by reference to physical constants in Table III. The outstanding feature is the increase in toxicity with increase in molecular weight.

Several investigators have reported that methyl alcohol is more[5, 16] or is less[8, 13, 17, 19, 33, 45, 55, 57] toxic than ethyl alcohol using acute criteria. Besides the above-mentioned investigations, textbooks and some of the literature leave the impression that methyl alcohol is more toxic than ethyl alcohol. Examination shows that this deduction is based upon the size of doses producing any untoward effect, regardless of the fact that the criteria may be dissimilar. The merits of each point of view are discussed later in this article. Studies have also been made in an effort to learn the reason why methyl alcohol, which is less toxic than ethyl alcohol when the production of death within the period of forty-eight hours is used as a criterion, is more toxic than ethyl alcohol when given in small doses over a period of time. The method of excretion, a subject still under investigation, seems to be responsible for this difference. Some investigators have concluded that any member of a homologous series is three times as toxic as its predecessor,[28, 49] but this exactness has been questioned in later work.[21, 58]

Iso-alcohols are usually considered to be less toxic than the normal straight-chain alcohols containing the same number of carbon atoms.[26, 29, 39, 48, 50, 51, 54] Tertiary alcohols are more completely narcotic than secondary alcohols and both are more so than primary alcohols.[26, 28, 29, 48] Temperature increases the toxicity and the narcotic action upon fish.[37] Intramuscular injections of methyl and ethyl alcohol into dogs and rabbits were reported to be a little more toxic than intravenous injections.[27] This conclusion, however, is based on relatively late deaths, not on experiments in which death followed promptly from the intravenous injection of relatively large doses. Other investigators[11, 15] have found subcutaneous injections to be less toxic than intravenous, intraperitoneal, or oral administration.

EXPERIMENTAL PROCEDURE

Alcohols.—The alcohols used in this investigation were procured from various commercial firms* as special C. P. alcohols. The boiling point and density of each was determined in the Analytical Reagent Investigations Laboratory† of this Bureau as soon as the products were received. The refractive indices at 25° C. were determined by one of us for another research (Table III). In most cases these constants agreed closely with data given in the literature and indicate that most of the alcohols were of a high degree of purity.

Rabbits.—The rabbits used in this investigation were bought on the open

*We wish to thank Carl O. Johns for the samples of secondary butyl alcohol and of secondary amyl alcohol (methyl n-propyl carbinol) supplied for this investigation.

†We wish to thank G. C. Spencer and R. M. Hann of the Analytical Reagent Investigations Laboratory for their cooperation in determining various physical constants.

TABLE III

PHYSICAL CONSTANTS OF ALCOHOLS USED

ALCOHOL	BOILING POINT AT 760 MM	DENSITY	INDEX OF REFRACTION AT 25° C. ND	FORMULA	MOLECULAR WEIGHT	RATIO OF MOLECULAR WEIGHTS ETOH—1.00)
Methyl	66.4*	0.7976 at 15	1.3275	$H—CH_2OH$	32.037	0.69
Ethyl	77.9*	0.7948 at 15	1.3595	$CH_3—CH_2OH$	46.058	1.00
n-Propyl	97-98	0.8126 at 15	1.3820	$CH_3CH_2—CH_2OH$	60.079	1.30
Isopropyl	80.9	0.7976 at 25	1.3768	$CH_3(CH_3)CHOH$	"	1.30
n-Butyl	116.6	0.8089 at 25	1.3970	$CH_3CH_2CH_2—CH_2OH$	74.100	1.61
Isobutyl	106.0	0.8068 at 15	1.3936	$CH_3(CH_3)CH—CH_2OH$	"	"
sec-Butyl**	98.0	0.8108 at 16	1.3942	$CH_3CH_2(CH_3)CHOH$	"	"
tert-Butyl	82.0	0.7859 at 25	1.3840	$CH_3(CH_3)(CH_3)COH$	"	"
Isoamyl	130.1	0.8095 at 25	1.4042	$CH_3(CH_3)CHCH_2—CH_2OH$	88.121	1.91
sec-Amyl**	119.0	0.8091 at 25	1.4050	$CH_3CH_2CH_2(CH_3)CHOH$	"	"
tert-Amyl	101.5	0.8134 at 15	1.4020	$CH_3CH_2(CH_3)(CH_3)COH$	"	"

* Corrected for barometric pressure.
** Racemic mixtures.

market from dealers in or near the District of Columbia. No attempt was made to segregate the animals according to weight, color, or sex. All the animals used weighed over 1000 grams, most of them weighing between 1500 and 2500 grams. Darenberg[12] found that rabbits weighing more than 2400 grams were often extremely resistant to intravenous injections of alcohol.

Upon receipt in the laboratory all rabbits were weighed, tagged, and placed in hutches. They were fed a mixture of alfalfa hay and oats daily, and carrots several times a week. Usually animals were held for a fore-period of ten days or two weeks before use, in order to make sure that they were in good condition. They were usually weighed three times a week and those in poor condition, as indicated by consistent loss in weight, were not used.*

Method of Administration.—The rabbits were weighed on the morning of an experiment and injections were usually made between 9 and 11 A.M. For the first few experiments about 50 c.c. of diluted aqueous alcohol solution per kilo body weight was injected. Subsequently the desired dose of alcohol was added to a sufficient volume of normal saline solution to make a total of 50 c.c. immediately before injection, whether or not complete solution could be obtained.

A vaselined rubber catheter (size 14 or 15 French) was used as a stomach tube. It was passed into the stomach of the rabbit and the diluted alcoholic solution or mixture was allowed to run into the stomach, either by gravity through a funnel which was immediately washed with a small quantity of

*Animals were used which were in good condition, since Mansfield[40] observed a difference in narcotic dose depending upon whether starved or well nourished animals were used. We have not investigated this phase.

water to wash in all the alcoholic solution or, later, forced directly into the stomach tube from a 50 c.c. record syringe.

Classification of Pharmacologic Effect.—Immediately after injection, the stomach tube was removed and the animal was placed on a large table. The time of injection and the appearance of symptoms grouped in accordance with the following criteria, were carefully recorded.

(1) *Staggering or Excitement.*—This stage was arbitrarily taken as the first stage of effect. The animal usually roamed wildly about (incoordination), and at times would plunge and attempt to jump from the table to the floor. The respiration and pulse were rapid.

(2) *Light Narcosis.*—Light narcosis followed the first stage when a large enough dose had been given. The animal usually sank into a stupor, ceased to move, and rested on its side or chest, making few if any voluntary movements. It would, however, respond to manual compression or stimulation of the thigh (muscles or sciatic nerve) by moving or by rising to its feet and moving away, but it would lie down again.

(3) *Profound Narcosis.*—Profound narcosis usually followed very rapidly after light narcosis if large enough doses were given. In this stage the corneal reflexes disappeared and the animal lay motionless, making no responses to a stimulus such as stimulation of the thigh. Occasionally, however, involuntary motions, such as attempted running or involuntary winking, after the disappearance of corneal reflexes, were noticed. Nystagmus was frequently observed. Respiration was diminished and labored. The rate of the heart beat was also diminished.

(4) *Death.*—Animals which had reached the stage of profound narcosis were inspected frequently to determine the approach of death, and all animals were inspected before the laboratory was closed in the evening. To prevent the possibility of death from exposure to low temperatures while narcotized, the laboratory temperature was maintained at about 65° or 70° in the winter by a constant temperature apparatus.

(5) *Recovery.*—After profound narcosis was produced, efforts were made in all cases to determine the first appearance of voluntary movements. If the animal was only in the light narcosis stage, the first appearance of an ability to assume a standing position was noted. In each case an attempt was made to determine when the animal was practically normal, as judged by its appearance, respiration, color of skin, powers of motion, and readiness to eat carrots, apples, or similar appetizing food.

RELATIVE MOLAR NARCOSIS AND TOXICITY

Basis of Comparison of Action of Alcohols.—Most of the data given in the literature deal with the production of death. In the investigation here reported, the quantities of the alcohols required to produce narcosis and to produce death within a period of approximately twenty-four hours were determined.

The minimum narcotic dose (M N D) was taken to be that dose by which light narcosis was produced in about half of the animals. For instance,

where injections of 5 c.c. of ethyl alcohol per kilo produced no narcosis and injections of 6 c.c. per kilo, the next larger dose, produced narcosis in all test animals fairly rapidly, the M N D was half way between these doses, 5½ c.c. per kilo. The certain lethal dose (C L D) was the dose which would produce death in about twenty-four hours in practically all animals injected. When a given dose produced death in only a portion of the animals within twenty-four hours and the next higher dose injected produced death in all animals, or practically all the animals, in half a day or less, that quantity half way between was arbitrarily selected as the C L D.

EXPERIMENTAL RESULTS AND DISCUSSION

The M N D and C L D are given in Table IV. The average values in cubic centimeters per kilogram are given in the second and third columns; the corresponding molar values are given in the next two columns. To facilitate comparisons of narcotic or toxic doses, the ratios of molar potency have been calculated, taking the absolute molar values of ethyl alcohol (0.0945 mol. per kilogram as the minimum narcotic dose and 0.2150 mol. per kilogram as the certain lethal dose) as units. The last column shows the ratios obtained by dividing the C L D by the M N D, arranged according to the alcohol group.

TABLE IV

NARCOTIC AND TOXIC POTENCIES OF ALIPHATIC ALCOHOLS. ORAL ADMINISTRATION TO RABBITS

ALCOHOL	AVERAGE C.C. PER KG.		MOL. PER KG.		RATIO OF MOLAR POTENCY ETOH —1.00		RATIO $\frac{\text{CLD}}{\text{MND}}$			
	MND	CLD	MND	CLD	MND	CLD	N-PRIMARY	ISO-PRIMARY	SECONDARY	TERTIARY
Methyl	7.5	18.0	0.1875	0.4500	0.50	0.48	2.4	–	–	–
Ethyl	5.5	12.5	0.0945	0.2150	1.00	1.00	2.3	–	–	–
n-Propyl	1.75	3.5	0.0236	0.0472	4.0	4.6	2.0	–	–	–
Isopropyl	2.85	10.0	0.0380	0.1333	2.5	1.6	–	–	3.5	–
n-Butyl	1.05	4.25	0.0115	0.0465	8.2	4.6	4.0	–	–	–
Isobutyl	1.75	3.75	0.0191	0.0410	4.9	5.2	–	2.1	–	–
sec-Butyl	1.25	6.0	0.0137	0.0655	6.9	3.3	–	–	4.8	–
tert-Butyl	1.80	4.5	0.0191	0.0480	4.9	4.5	–	–	–	2.5
Isoamyl	0.875	4.25	0.0080	0.0391	11.8	5.5	–	4.9	–	–
sec-Amyl	0.50	3.5	0.0046	0.0322	20.5	6.7	–	–	7.0	–
tert-Amyl	0.75	2.5	0.0069	0.0230	13.7	8.6	–	–	–	3.3

MND—Minimum Narcotic Dose.
CLD—Certain Lethal Dose.

As the molecular weight increases, the narcotic and toxic properties increase; the narcotic properties for most alcohols increased more rapidly than the toxic properties. As the homologous series of normal, iso-primary, secondary, or tertiary alcohols, is ascended, the lethal dose in any series becomes a greater multiple of the narcotic dose, with the exception of methyl and normal propyl alcohol. Methyl alcohol has always been an exception in making generalizations,[5, 55] both chemical and pharmacologic. Normal propyl alcohol is the only primary alcohol with which we are familiar that is miscible in all proportions with both oil and water.

Experiments with iso-alcohols have been very limited. Because "isopropyl" alcohol has the name "iso," is not a criterion that it should be considered in the series with primary isobutyl and isoamyl alcohols. It is a secondary alcohol and being the first member in such a series probably explains why it exhibits irregularities. Therefore, we have results on only one alcohol (isobutyl) suitable for comparison with those obtained on a normal alcohol of the same carbon content. Alone, the data upon this alcohol are not sufficient to warrant drawing definite conclusions. Our results with this lone alcohol show that it is less narcotic but more toxic than its straight chain isomer. This does not agree with expectations.[26, 29] Further work is necessary.

The secondary alcohols show the greatest ranges between their narcotic and toxic doses. These alcohols are more narcotic than the isomeric isoprimary, but less narcotic than the normal primary alcohols used. They are also less toxic than the normal alcohols used.

The data on the tertiary alcohols are insufficient for a prediction on the behavior of the series. Tertiary butyl is about as toxic and narcotic as the isoprimary butyl compounds, whereas tertiary amyl is a little more toxic and narcotic than its corresponding isomer.

Methyl Alcohol.—Deaths from alcohols other than methyl occurred within twenty-four hours in a great proportion of cases. Methyl alcohol intoxication from a single dose is clearly divisible into two classes, (1) acute poisoning, and (2) delayed poisoning, in which, while the animal is not sober, it is more or less completely exhausted. An animal's degree of narcosis as observed in 1, distinctly lessens before it dies in stage 2. Since in our experiments other alcohols have not shown a delayed poisoning of the same type as methyl alcohol, it is manifestly unfair to make a comparison except on the common basis of acute effect. However, in our experiments, irrespective of class, considering *all* deaths, methyl alcohol appears to be as toxic as ethyl on the cubic centimeter basis; on the molar basis, ethyl alcohol would then be, of course, 33 per cent more toxic than methyl.

From the above consideration, which concerns acute intoxication, we do not wish to leave the impression that the other effects are to be minimized. From a practical standpoint any untoward effects whatsoever, chronic or delayed poisoning, blindness, cystitis, etc., are to be considered. The purpose of the comparison, therefore, underlies the analysis, and necessarily narrows the applications of the conclusion. It is hardly necessary to state that alcohols other than methyl, using different experimental conditions, might give results which we could not compare with methyl on account of the lack of a common basis.

CONCLUSIONS

The general statement regarding the increase in toxicity in homologous series with increase in molecular weight has been verified for oral administration.

This law may be extended to narcotic action following oral administration.

Increase in narcotic activity is more rapid than increase in toxicity in an

homologous series of alcohols, when administered orally, some alcohols having greater narcotic potency than might be expected from their molecular weights.

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A STUDY OF TISSUE AUTOLYSIS IN VIVO*

III. Observations Using the Spleen

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WE have demonstrated[1] that the autolysis of a small amount of liver tissue free within the abdominal cavity is accompanied by definite and constant changes in the animal's blood chemistry. We have also[2] compared the toxicity of normal liver tissue and autolyzed liver tissue by administering saline extracts of such tissues intravenously.

It has been the object of the present work to ascertain whether or not tissues, other than liver, were capable of liberating toxic material of sufficient potency to kill the animal. For this work the spleen was selected as a tissue of choice since its removal is accompanied by such slight physiologic disturbance and it also has the additional advantages of being readily accessible and easy to remove.

METHOD

All operations were performed in the evening under aseptic conditions, using ether anesthesia with a preliminary injection of morphine sulphate ¼ gr. and atropine sulphate 1/100 gr., the animals having been fed from eight to ten hours prior to the operation. Briefly, the operation consisted of clamping off the spleen through its attachment, sectioning the attachment between the spleen and clamp, ligating the cut surface by use of a figure-of-eight suture, weighing the spleen and returning it to the abdomen. The operation usually required from fifteen to twenty minutes, and the spleen varied in weight from 23 to 77 grams.

None of the animals showed signs of surgical shock following the operation, and the following morning there was but very little evidence of trauma. The animals seemed to withstand the operation even better than those in which a portion of the liver was sectioned. Half of the animals of this series died, and such animals displayed all the characteristics of animals dying from liver autolysis. However, death never occurred so soon following operation and the symptoms were considerably less pronounced.

Postmortem examination revealed essentially the same findings as described in the study of liver autolysis. The abdomen usually contained 100 to 300 c.c. of brown colored fluid, but in no case did the fluid appear to be actual blood. A pseudofibrinous exudate was usually present, being different from a true fibrinous exudate in that it was easily removed, leaving a smooth shiny surface. The intestines were generally hyperemic with marked congestion.

*From the Henry Ford Hospital, Detroit, Mich.

The omentum was firmly wrapped about the sectioned spleen, the omentum being markedly hemorrhagic and discolored. The autolyzing spleen ranged from dull to a bright green in color. The spleen showed the same autolytic changes which accompany liver autolysis, being less firm than normal spleen as well as gas containing. There was a marked loss in weight of the sectioned spleen, such loss ranging from 13 to 20.5 grams.

We realize that our study of six cases is not extensive enough to warrant any sweeping conclusions. However, unexepected conditions have developed which make it necessary to postpone indefinitely any further work on the subject. We have, therefore, thought it advisable to offer the work in its present state of development.

THE RELATION BETWEEN THE WEIGHT OF THE SECTIONED SPLEEN AND THE SIZE OF THE ANIMAL AND THE PERIOD OF LIFE EXPECTANCY

In our observations on liver autolysis we noted that the weight of the liver sectioned did not give any definite indication as to the period of life expectancy. We then expressed the opinion that the length of life following sectioning of a portion of the liver depended upon two factors: (1) the rate of liberation of the toxic material, and (2) the rate of absorption of the toxic substance. Our work with the spleen has led us to the same conclusion, which is based upon Table I.

TABLE I

WEIGHT OF ANIMAL	WEIGHT OF SPLEEN	PERIOD OF LIFE AFTER OPERATION
16.8 K	68 Grams	– – –
11.0 K	23 grams	45 hours
13.1 K	41 grams	36 hours
15.9 K	77 grams	36 hours 25 minutes
21.2 K	56 grams	– – –
23.1 K	42 grams	– – –

BLOOD CHEMISTRY

The following blood constituents were determined: 1. nonprotein nitrogen, 2. urea, 3. amino acid, 4. uric acid, and 5. sugar. The three animals which lived following the sectioning of the spleen did not show any marked changes in their blood chemistry such as occurred in the animals dying from liver autolysis. This point is illustrated by the blood urea and amino acid fractions.

TABLE II

UREA NITROGEN BEFORE AND AFTER OPERATION

DOG		BEFORE	12 HR.	24 HR.	36 HR.	48 HR.	60 HR.	72 HR.
No.	14	21.	16.3	15.4		14		10.7
No.	22	18.2	10.3	10.7	10.7	12.6		10.7

TABLE III

AMINO ACID NITROGEN BEFORE AND AFTER OPERATION

DOG		BEFORE	12 HR.	24 HR.	36 HR.	48 HR.	60 HR.	72 HR.
NO.	14	8.52	6.82	5.98		5.78		5.71
NO.	23	7.45	6.09	7.09		6 90		
NO.	22	7.00	7.00	7.82	7.00	7.00		7.00

We have made complete chemical studies only on one animal which died following the sectioning of the spleen. The animal showed no marked changes in any of nonnitrogenous blood constituents except the amino acid fraction which is represented by the figures in Table IV.

TABLE IV

AMINO ACID NITROGEN BEFORE AND AFTER OPERATION

DOG	BEFORE	12 HOURS	24 HOURS	36 HOURS
NO. 21	7.27	7.78	8.19	DEAD

This one case is not sufficient to warrant a definite conclusion, but it is suggested that either the other nonprotein nitrogen constituents are not increased or that if an increase occurs it does so in the last twelve hours of life.

Analysis of the abdominal fluid present at death occurring from spleen autolysis gave the figures shown in Table V.

TABLE V

SAMPLE	N.P.N.	UREA	URIC ACID	AMINOACID
DOG 21	58.8 – 60.	33.6 – 34.	2.32 – 2.35	
DOG 21	60.6 – 60.6	35.		12.7 – 12.7

It will be noted that the figures do not vary greatly from those which we have previously found associated with liver autolysis.

CONCLUSIONS

1. Autolysis of the spleen free within the abdominal cavity has been accompanied by death in 50 per cent of the animals studied.

2. Death from spleen autolysis has not followed so soon after operation as death from liver autolysis, the earliest death being thirty-six hours after sectioning the spleen.

3. It is suggested that the spleen either does not undergo as rapid autolysis as the liver tissue or that the toxic material liberated is not so potent.

4. Apparently the blood chemistry is but little altered in the animals which survive sectioning of the spleen, while those dying following the operation show some changes.

5. The analysis of the abdominal fluid present at death due to spleen autolysis shows the nonprotein nitrogen constituents are essentially the same as in those cases of death due to liver autolysis.

REFERENCES

[1]Mason, and Davidson: A Study of Autolysis in Vivo. I. Blood Changes, Physical and Chemical, JOUR. LAB. AND CLIN. MED., May, 1925, x, 622.

[2]Mason and Davidson: A Study of Autolysis in Vivo. II. A Pharmacologic Study of the Toxic Material, JOUR. LAB AND CLIN. MED. (In press).

THE EFFECT OF POTASSIUM OXALATE ON BLOOD SUGAR DETERMINATIONS*

By Henry J. John, M.D., Cleveland, Ohio

IN our routine work with diabetic patients, I have noticed an occasional low blood sugar figure which seemed out of proportion with the daily blood sugar estimations obtained from the same patient. In searching for a possible reason for this apparent discrepancy, I noticed that this low figure was obtained in those cases in which only a small quantity of blood—from ¾ to 1 c.c.—was taken, as in children. I, therefore, began to suspect that variations in the amount of the potassium oxalate, used to prevent clotting of the blood, might provide the explanation.

I believe that the usual practice in making blood sugar determinations is to put a pinch of oxalate into the test tube which is to receive the blood, the tube being well shaken as soon as the blood is added to insure thorough mixing, and the blood examined as soon as possible thereafter. Our own practice is to make the blood sugar determination within fifteen minutes.

In order to determine the effect of the oxalate on the blood sugar determination, the following experiment was carried out. Two specimens of blood equal in amount were taken from each of two patients and placed in test tubes. To one specimen from each patient, 15 to 30 mg. of potassium oxalate, the amount used in routine work, was added and to the other specimen from 250 to 350 mg. Blood sugar estimations on these mixtures as obtained by the Myers' modification of the Benedict method, gave the results shown in Table I.

Table I

SPECIMENS FROM PATIENT	SMALL AMT. OXALATE	LARGE AMT. OXALATE	VARIATION
Number	Blood sugar mg. per 100 c.c.	Blood sugar mg. per 100 c.c.	
1.	122	82	33%
2.	93	60	35%

These variations led me to make a further investigation of the possible effects of varying amounts of oxalate on blood sugar estimations. The following procedure was used: To each of two sets of eight test tubes containing respectively 15, 30, 45, 60, 75, 100, 150, and 200 mg. of potassium oxalate, 2 c.c. of blood was added. The blood added to the tubes in one set was taken from a diabetic patient and to those in the other set from a nondiabetic patient. Each tube was thoroughly shaken three times at intervals of from one to two minutes, after which whole-blood sugar estimations were made by the Myers-Benedict method with the fairly constant results shown in Tables II and III. The ex-

*From the Cleveland Clinic.
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periment was repeated using the Folin-Wu method of blood sugar determination, with the results shown in Tables IV and V. Table VI shows that the results obtained by the Myers-Benedict and Folin-Wu methods were fairly uniform.

These experiments show that the quantity of potassium oxalate used bears a definite relation to the blood sugar determination. As the amount of oxalate is increased, the blood sugar content determination is decreased in both diabetic and nondiabetic cases.

TABLE II

A. BLOOD SUGAR VALUES IN NONDIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Myers' modification of Benedict method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1) 15	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200	
Case 1	105	97	94	91			60	60	Blood
2	119	116	116	106	104	102	74	53	sugar
3	108	106	106	102	94	92	74	68	mg./100
4	95	95	94		70		60	30	c.c.
5	107	104	98	98		91	77	50	
6	110		104	97	92	87	81	75	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1	7	10	13			43	43	
2	2	2	11	12	14	38	55	
3	2	2	4	13	15	31	37	
4	0	1		26		37	68	
5	3	8	8		15	28	53	
6		5	12	16	21	26	32	
Average Loss	2.8	4.66	9.6	17	16.37	34	48	Per cent

TABLE III

A. BLOOD SUGAR VALUES IN DIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Myers' modification of Benedict method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1) 15	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200	
Case 1	258	227	212	207	193	192	187	143	Blood
2	348	344	336	313	309	277	224	191	sugar
3	405		394		333		333	263	mg./100
4	157		145		138		97	76	c.c.
5	150	150	148	143	141	132	128	116	
6	161	161	156	156	156	154	143	134	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1	12	18	20	25	26	28	44	
2	1	3	10	11	20	36	45	
3		3		18		18	35	
4		8		12		38	52	
5	0	1	5	6	12	15	22	
6	0	3	3	3	4	11	17	
Average Loss	3.25	6	9.5	12.5	15.5	24.3	36	Per cent

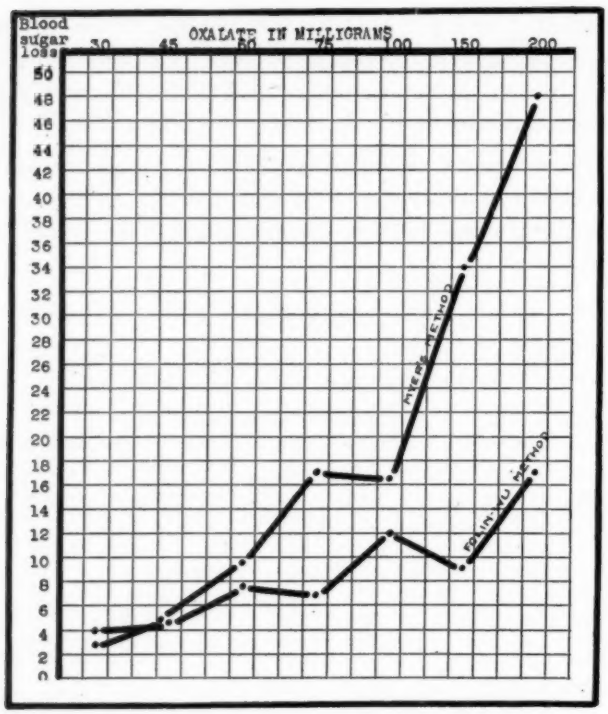

Chart 1.—Percentile decrease in the blood sugar content produced by varying amounts of potassium oxalate. Nondiabetic cases.

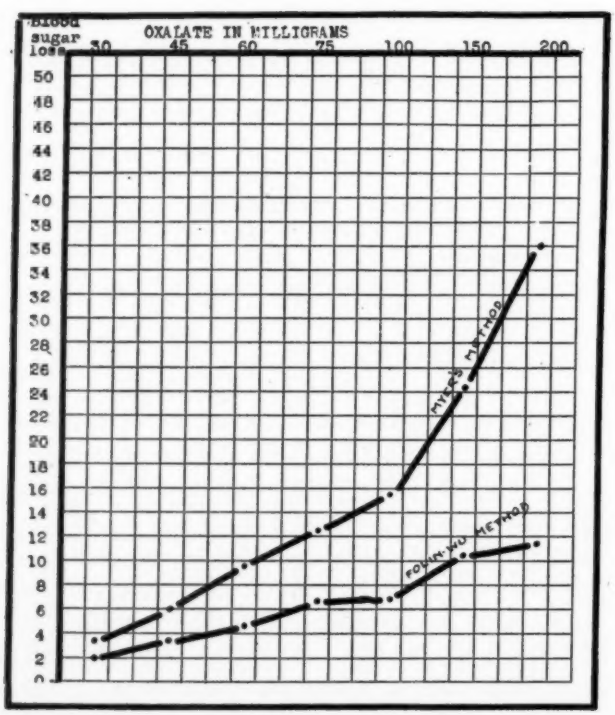

Chart 2.—Percentile decrease in the blood sugar content produced by varying amounts of potassium oxalate. Diabetic cases.

The explanation of this effect of potassium oxalate on the blood sugar determination must be left to the physiologic chemist. As a clinician, my interest lies in its practical application to our everyday work. It follows that in order to secure uniform and accurate results, the amount of oxalate used must be standardized. The need for such a standardization is indicated also by the variation in the amounts recommended by different authors. Thus, in the United States Army Manual and by Mathews, 50 mg. of oxalate per 10 c.c. of blood is recommended, while Folin, Cummer and Stitt advise 20 mg.

Because of this discrepancy, I made an investigation to see how large a quantity of blood could be kept from clotting by 15 mg. of oxalate. I set up a series of test tubes, each containing 15 mg. of oxalate, and varied the amount of

TABLE IV

A. BLOOD SUGAR VALUES IN NONDIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Folin-Wu Method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1) 15	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200	
Case 1	139		139		131		131	121	Blood
2	116	111	110		110		108	101	sugar
3	108	100	95	95	91	91	91	66	mg./100
4	122		118		117		114	113	c.c.
5	106	105	103	103	103	98	97	92	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1		0		6		6	13	
2	4	5		5		7	13	
3	7	12	12	16	16	16	39	
4		3		4		7	7	
5	1	3	3	3	8	9	13	
Average Loss	4	4.6	7.5	6 8	12	9	17	Per cent

TABLE V

A. BLOOD SUGAR VALUES IN DIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Folin-Wu Method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1) 15	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200	
Case 1	325			323		323		286	Blood
2	175	165	158	152	152	152	148	142	sugar
3	351		339		339	328		323	mg./100
4	155	155	155	155	146	146	144	142	c.c.
5	182	182	182	174	174	172	166	166	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1			1		1		12	
2	6	10	13	13	13	15	19	
3		4		3	7		8	
4	0	0	0	6	6	7	9	
5	0	0	4	4	6	9	9	
Average Loss	2	3.25	4.5	6.5	6.6	10.33	11.4	Per cent

TABLE VI

AVERAGE PERCENTILE LOSS OF BLOOD SUGAR
(Due to varying amounts of Oxalate)

NONDIABETIC CASES

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200
Percentile loss Folin-Wu Method	4	4.6	7.5	6.8	12	9	17
Percentile loss Myers' Method	2.8	4.66	9.6	17	16.37	34	48

DIABETIC CASES

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200
Percentile loss Folin-Wu Method	2	3.25	4.5	6.5	6.6	10.33	11.4
Percentile loss Myers' Method	3.25	6	9.5	12.5	15.5	24.3	36

TABLE VII

THE AMOUNT OF BLOOD WHICH 15 MG. OF POTASSIUM OXALATE WILL KEEP FROM CLOTTING

Case 1					
Pot. Oxalate mg.	15	15	15	15	15
Blood c.c.	2	4	6	8	10
Clotting	neg.	neg.	neg.	neg.	neg.
Blood Sugar mg./100 c.c.	90	90	90	90	90
Case 2					
Pot. Oxalate mg.	15	15	15	15	
Blood c.c.	1	3	8	15	
Clotting	neg.	neg.	neg.	clotted	
Blood sugar mg./100 c.c.	113	113	113	113	

blood put into each, with the results shown in Table VII which demonstrates that when 10 c.c. of blood or less is used, 15 mg. of oxalate is sufficient to prevent clotting. Since the variations in the blood sugar estimation are small when the amount of oxalate varies between 15 and 30 mg., it would seem that for routine work it would be sufficient to place 15 mg. of oxalate in a test tube and use this as a guide for the amount to be placed in each tube used. It would not be practical and, as I have shown, it is unnecessary, to weigh each 15 mg. portion accurately, as that is time-consuming, and the error occurring from the slight increase, is hardly worth consideration. It is the use of the considerably larger portions of oxalate which needs to be eliminated. If all physicians and clinicians will adopt such a uniform basis, our figures will be more accurate and can be used fairly for comparative studies. There are many small technical problems connected with the study of diabetes, and it is only by solving each of these that we can properly evaluate our findings.

A POSSIBLE SOURCE OF ERROR IN THE USE OF RUSSELL'S DOUBLE SUGAR MEDIUM*

By William P. Belk, M.D., and Margaret Duff, Bryn Mawr, Pa.

IT has been the practice in this laboratory, and we believe elsewhere, in searching feces and urine for typhoid bacilli, to fish characteristic colonies from Teague's, or Endo, plates directly to Russell's double sugar tubes. If the reaction on this medium was characteristic, especially if there was no production of gas, further identification was undertaken. Otherwise a negative result was reported.

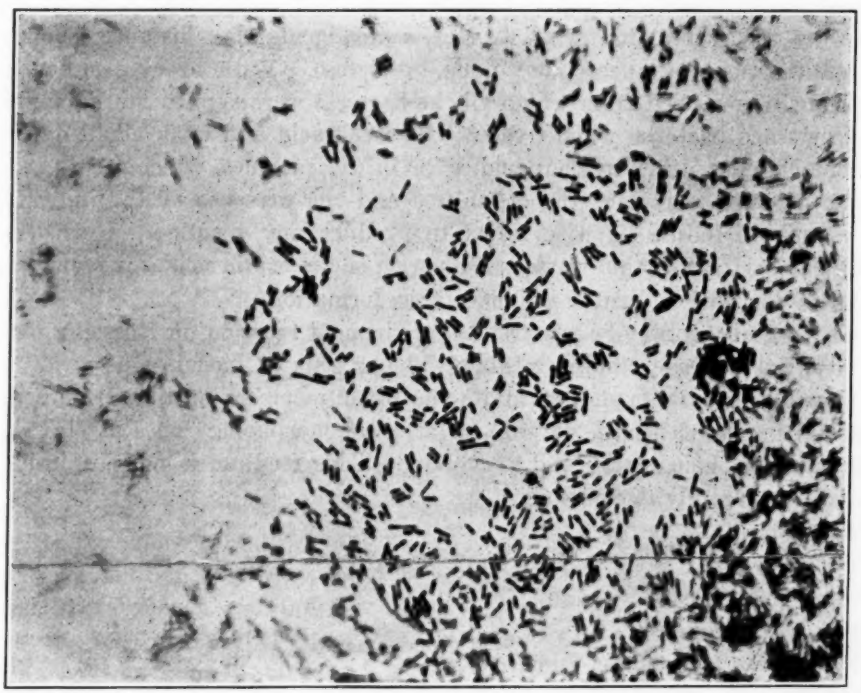

Fig. 1.—B. typhosus from Russell's double sugar medium showing vacuoles.

Before making subcultures, or agglutination tests, Gram's stains were naturally studied from the growth on Russell's slants to determine purity of culture and typical morphology. It was in this step that our error occurred. It was found that typhoid bacilli, as well as other members of the Typhoid-Colon-Dysentery group, appeared, under these conditions, as large bacilli with vacuolated ends. The appearance was quite closely that of a spore-forming organism. Moreover, when litmus was the indicator in the Russell's medium,

*From the Laboratory of The Bryn Mawr Hospital, Bryn Mawr, Pennsylvania.
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ordinary decolorization in some instances left a bluish tinge in the bacteria, which gave them a Gram-positive appearance. This undoubtedly resulted in discarding cultures which merited complete identification.

This reaction was further studied with the following organisms: A stock strain of B. typhosus (U. S. Army); three cultures of B. typhosus from feces of a typhoid patient, identified by the specific agglutination; two strains of B. fecalis alcaligenes, and B. dysenteriae, B. Shiga (Stock). These were inoculated into the following sugar media; Russell's double sugar with litmus indicator; Russell's double sugar with Audrade's indicator; Teague's plates; and bouillon containing 1 per cent of glucose, lactose, saccharose, xylose, arabinose, mannite, maltose, dulcite, salicin, and inulin. All of the nine organisms showed well marked vacuolization on both Russell's tubes, and on Teague's plates. The stock strain of B. typhosus was vacuolated in every variety of sugar media. The other organisms were studied from the glucose and lactose bouillon only. Here the reaction was variable, some organisms showing many vacuoles, others none. This variation did not correspond at all to fermentation of the different sugars by the several bacteria; B. fecalis alcaligenes, for instance, showing a marked reaction in glucose, but none in lactose. With every series a plain agar slant was carried from which all the bacteria were typical morphologically.

Unstained bacteria, when treated with weak acid and weak alkali developed no color changes either in their bodies, or in the vacuoles, which would seem to eliminate vital staining of the organisms and the presence of the indicator in the vacuoles. Iodine likewise failed to produce any reaction. However, the definite association of this vacuolization with sugar media makes it seem reasonable that the carbohydrates influence this formation.

Incidentally it may be remarked that the acid reaction on Russell's medium was atypical in some respect in about 50 per cent of inoculations.

Conclusion: B. typhosus, and some other members of the Gram-negative intestinal group of bacilli, develop an atypical morphology on Russell's double sugar medium, being large and vacuolated. This medium is not a suitable one from which to study morphology.

LABORATORY DIAGNOSIS IN THROAT INFECTIONS*

II. The Direct Smear as an Aid to Diagnosis

By C. C. Young, Dr. P. H., and Minna Crooks, Lansing, Michigan

As indicated in a previous communication,[1] the routine use of the swab for transporting throat specimens permitted the preparation of a direct smear for immediate examination. We have found this procedure valuable in making a differential diagnosis and in saving time, as illustrated in Table I.

Table I

Direct Microscopic Examinations on Diagnostic Swabs
1923

Clinical Diagnosis		Laboratory Findings		
		Direct Smear		Serum Media
		K. L.	V. A.	K. L.
Diphtheria	148	102	14	134
Septic sore throat	36	20	10	26
Vincent's angina	10	2	8	2
Tonsillitis	43	11	4	39
Doubtful	74	20	22	52
	311	155	58	253

K.L.—Klebs-Loeffler bacilli. V.A.—Organisms of Vincent.
(A comparison of the laboratory and clinical findings on positive diagnoses of three months in 1923.)

In Table I it may be observed that 155 swabs showed diphtheria bacilli and 58 showed organism of Vincent's on direct smear. Of 263 swabs found positive on culture, 155 (over 61 per cent) were found positive on direct examination. The early differential diagnosis made it possible to establish quarantine and immunize contacts eighteen to twenty-four hours earlier than otherwise in an appreciable number of cases.

The time factor is important in estimating the value of the direct microscopic examination in throat infections, but of much greater importance is the diagnosis of Vincent's angina, for it may be mistaken for diphtheria, as pointed out by Reckord and Baker[2] in a study of 56 cases. The incidence of Vincent's angina has no doubt been raised by the returned soldiers but on account of the lack of differential diagnosis, cases are missed. Organisms of Vincent were found on 5.7 per cent of the diagnostic swabs in the laboratories of the Michigan Department of Health during the fiscal year ending June 30, 1923, and were found in many cases which were clinically confused with diphtheria, as shown in Table I.

STATUS OF THE DIRECT MICROSCOPIC EXAMINATION

Since 1884, when Loeffler's cultural findings superseded Kleb's observations on morphology, the value of the direct smear in diphtheria diagnosis has

*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan. Received for publication, November 29, 1924.

been questioned. Many authorities of today do not discuss it and those who do, recommend it with reservations. Park[3] recommends its use by experienced bacteriologists and bases a positive finding on contour, mode of division, and arrangement. Stitt[4] states that an immediate diagnosis is possible in 36 per cent of the cases and recommends Gram's method of staining. Osler in his textbook *"Practise of Medicine"* recommends an examination of a cover-glass preparation from membrane by an experienced bacteriologist as a confirmation of clinical diagnosis and the means of differentiating between pseudo and true membranes. He does not, however, advise its use by clinicians. Novy[5] men-

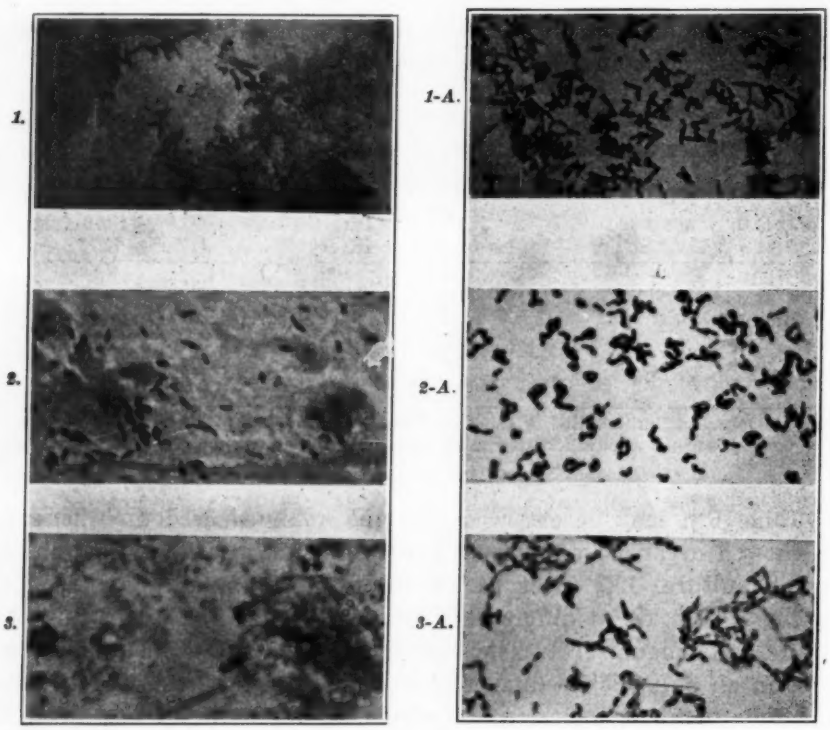

Plate 1.—Diphtheria bacilli in direct smears and in serum media. *1, 2, 3.* Direct slide preparations stained with alkaline methylene-blue. *1-A, 2-A, 3-A.* Smears of cultures from the swabs *1* and *3* respectively. *2.* Direct slide preparation from swab of sore on cheek stained with alkaline methylene-blue. *2-A.* Smear of culture of swab 2 stained by Gram's method. All cultures were pathogenic for guinea pig.

tions the cover-glass preparation from the membrane stained with methylene-blue as a method of procedure but does not recommend it.

Bleyer[6] discusses direct smears in diphtheria from the point of view of the clinician. In his investigation he was able to demonstrate diphtheria bacilli in 59 per cent of direct microscopic examinations made on exudates collected with a special "scoop" from known cases of diphtheria. He cites the frequent finding of Vincent's angina coexistent with diphtheria. Bleyer uses Greenthal's method of staining and recommends the direct smear as an aid to diagnosis if the clinician "seeks those forms with which he is familiar and trusts his judgment to no others."

SIGNIFICANCE OF MICROSCOPIC FINDINGS

The variability in the morphology of diphtheria organisms is an established fact. Westbrook, in 1900, classified diphtheria-like organisms morphologically and designated nineteen different types. He divided them into virulent and nonvirulent based upon his findings which showed that certain types were constant in diphtheria. Albert,[7] in 1920, used a differential staining method and classified them by their staining reaction into eight types. He found that cultures showing no granular forms were nonvirulent but did not find that all cultures showing granules were virulent. Laybourn, in 1921,[8] found morphology to be affected by the P_H value of the medium and Albert[9] in the same year re-

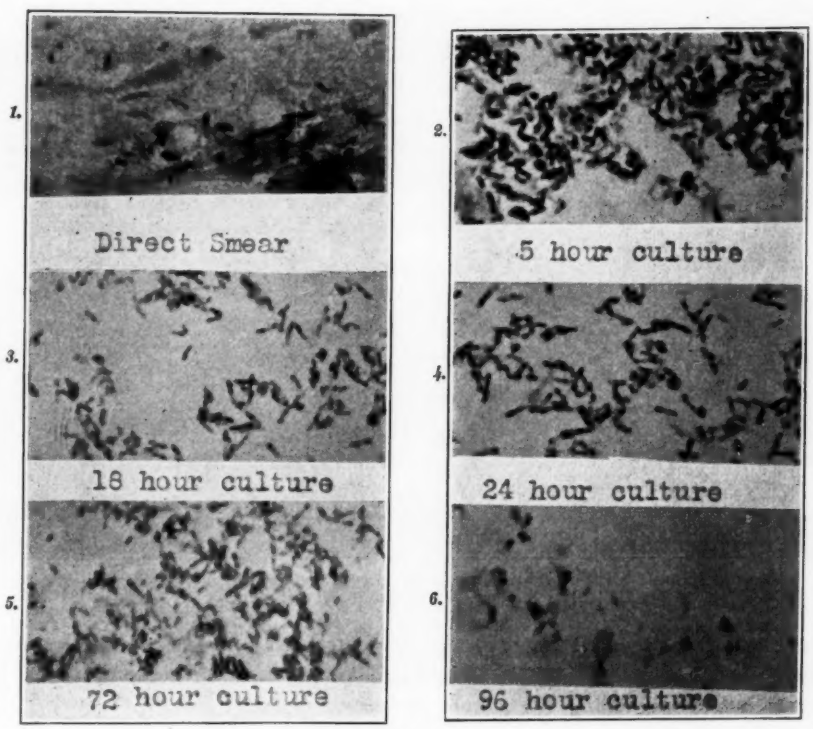

Plate 2.—Diphtheria bacilli stained with Loeffler's alkaline methylene-blue. *1.* Stained preparation from swab. *2, 3, 4, 5, 6.* Stained preparation from culture at different periods of incubation.

ports variations attributable to age of culture. Our experience with cultures on serum media mailed into the laboratory for virulence test has demonstrated the latter fact. Cultures on Loeffler's serum medium showing no typical bipolar staining organisms on arrival induced toxemia and death in guinea pigs when inoculated by the whole culture method. Stovall, in 1923,[10] studied the influence upon morphology and staining of B. diphtheria by growth in mixed cultures and found that the change was so marked as to completely alter the classification of the diphtheria bacilli when Westbrook's classification was used. It would seem from this brief survey that the recognized variations in the morphology of diphtheria bacilli in the stained slide preparations from throat swabs are attributable to (1) reaction and constituents of the medium supplied

by the mucous membrane of the throat; (2) growth in symbiosis with other organisms; and (3) age of organisms. Consequently, we observe on the direct smear the solid type and the clubbing distal ends of the young cultures (Plates 1 and 2), the metachromatic granules of the routine culture, or the pleomorphism of the old culture when the throat furnishes optimum conditions of growth, depending on the period of incubation, temperature, and P_H of the pabulum.

Freshly collected material from the throat no doubt has the greatest diagnostic value. Swabs one to three days in the mails, however, routinely give in these laboratories positive findings in both diphtheria and Vincent's angina. The procedure is in brief as follows:

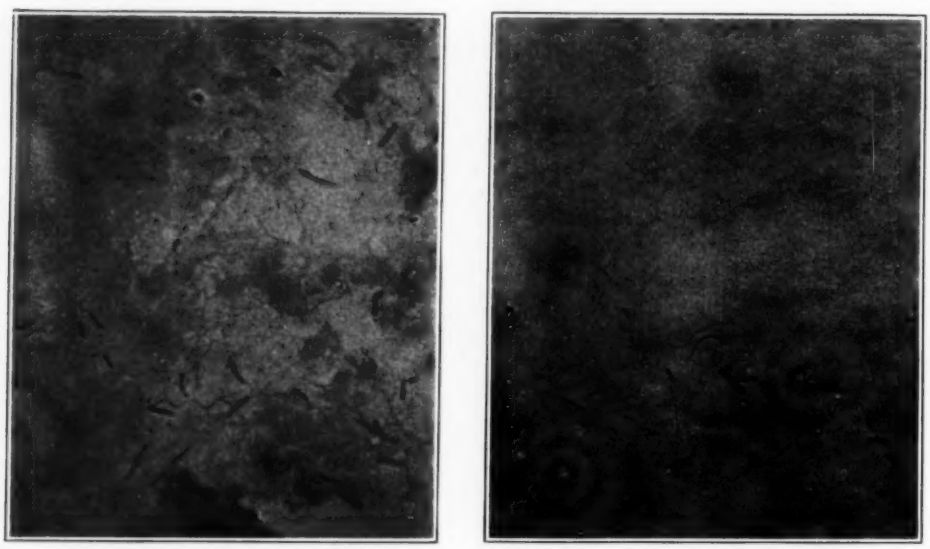

Plate 3.—Photographs of slide preparations from throat swabs stained with alkaline methylene-blue showing organisms of Vincent.

*1. First, inoculate blood plate; second, inoculate Loeffler's serum; make smear on slide.

NOTE: Only when freshly collected specimens are submitted are the slide preparations made before inoculating the culture mediums.

2. Examination of direct slide preparations.
   a. Stain with Loeffler's methylene-blue.
      To stain: Dry and fix preparation. Flood slide with stain for two minutes, wash in water, dry in air.
   b. Report only positive findings on direct smears. If a report is requested, give only a provisional negative report and make a final report from the culture.
   c. Report organisms of Vincent when B. fusiformis is found in the presence of spirocheta. (See Plate 3.)
3. General Ruling.
   a. At 8:00 A. M. daily of after ten to 18 hours incubation, spread smears, stain with Loeffler's alkaline methylene-blue and examine.
   b. Any cultures which show solid forms (A2 and C2) or overgrowth of other organisms should be smeared again and stained by a differential stain to demonstrate granules. Cultures showing granules and doubtful morphology should be stained by Gram's method.

*From the direction sheet of the Bureau of Laboratories, Michigan Department of Health.

We routinely use alkaline methylene-blue for two reasons: (1) the various differential stains do not stain the young culture which is one of the principle diagnostic features of the microscopic picture in direct smears; (2) we do not feel that the experienced microscopist needs differential stain in order to identify diphtheria bacilli. For, since we make our identification of diphtheria bacilli upon characteristic morphology, arrangement and bipolar staining, we want a stain that will give us a clear-cut picture of arrangement and morphology.

SUMMARY

A report of the findings on direct examinations of throat swabs is presented. Smears made from swabs mailed into the laboratory showed diphtheria-like bacilli on about 60 per cent of the positive cultures. The organisms of Vincent were found in many cases which were clinically confused with diphtheria. Smears from both the swabs and cultures were stained with Loeffler's alkaline methylene-blue and a diagnosis made upon staining reaction, morphology, and arrangement. Microphotographs are submitted, showing the effect of incubation period on morphology and the similarity of the microscopic picture in direct smear and in the young culture.

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CONTRIBUTING CAUSE OF TURBIDITY OF NESSLERIZED SOLUTIONS IN THE DETERMINATION OF UREA IN THE WHOLE BLOOD*

By Frank B. Cooper, Trudeau, N. Y.

IN the determination of urea in blood during the past few years, we have noticed along with other observers, that turbidity frequently developed after nesslerization of the unknown solution.

The technic employed is that described by Gradwohl and Blaivas[1] and offers the advantages of speed, accuracy and ease of manipulation. This method is practically the same as that of Van Slyke and Cullen[2] except that the final ammonia solution instead of being titrated, is nesslerized as in Folin and Farmer's[3] method for total nitrogen in urine.

It was found, however, that a certain number of determinations failed, on account of the development of turbidity shortly after addition of Nessler's reagent to the final ammonia solutions. In no instance did the standards give turbid solutions.

After carefully checking over the various reagents used, the cause was traced to the small amount of caprylic alcohol, added to prevent foaming, which was carried over with the ammonia during aeration. The degree of turbidity produced varies directly, and the time of turbidity inversely with the amount of alcohol used.

Fresh samples of caprylic alcohol manufactured by three different companies were next tried and yielded practically identical results.

It was found that 0.02 c.c. of caprylic alcohol prevented foaming for twenty-five minutes, and gave solutions which remained clear for eight hours after addition of Nessler's reagent. Four-hundredths cubic centimeter prevented foaming for forty minutes and gave solutions remaining clear for one-half hour. Amounts over this prevented foaming for longer periods, but caused turbidity in ten to fifteen minutes.

Various antifoaming agents were next tried, some of which gave even greater turbidity than caprylic alcohol. The best was found to be a mixture of 1 c.c. of toluol and 0.02 c.c. of caprylic alcohol. This mixture prevented foaming for at least forty-five minutes, ample time for the aeration, and gave nesslerized solutions which remained clear for at least one hour.

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CLINICAL STUDIES ON THE KAHN REACTION FOR SYPHILIS*

II. Specificity of Test

By Harther L. Keim, M.D., Ann Arbor, Mich. and R. L. Kahn, Sc.D., Lansing, Mich.

A TEST for syphilis should conform with two major requirements. It should possess a high degree of sensitiveness in detecting the presence of syphilis and it should possess specificity, so that a positive reaction will be indicative of no pathologic condition except syphilis.

The sensitiveness of the Kahn precipitation test has been considered in the first paper of this series.[1] The test was compared with two separate Wassermann systems, one of conservative sensitiveness (one hour fixation) and one of high sensitiveness (overnight fixation). The cases of syphilis studied totaled 625. These included: primary, 25; secondary, 64; tertiary, excluding cerebrospinal, 87; cerebrospinal, 192; latent, 218; congenital, 39. It was observed that the diagnostic value of the Kahn test approximated in its results the highly sensitive Wassermann test rather than the one of conservative sensitiveness.

This study was undertaken to find to what degree the Kahn test is specific for syphilis alone. We, herein, present the results of examinations of 2,500 nonsyphilitic cases at the University of Michigan Hospital.

THE KAHN TEST IN NONSYPHILITIC CASES

As in the first of this series of studies, the blood from each case was examined with the Kahn precipitation test and with two separate Wassermann tests. In each of these two tests a cholesterinized antigen was employed with ice-box fixation; one using an eighteen hour and the other a one hour fixation period. In the case of the Kahn test, no incubation was employed, the results being read immediately after mixing the ingredients.[2, 3]

Of the 2,500 cases studied, negative results with all three methods were obtained in 2,490 cases. These cases include: dermatologic, 750; medical, 495; surgical, 498; gynecologic, 151; otologic, 183; ophthalmologic, 235; and neurologic, 178. A classification of these cases is given in Table I. Positive reactions with one or more of the methods were obtained in the remaining ten cases. Of this number, the Kahn test gave seven reactions varying from one to three plus, representing an error of twenty-eight hundredths of one per cent. The findings are summarized in Table II.

DISCUSSION OF RESULTS

Of special interest in these results is the finding that the Kahn test gave no false reactions in the febrile diseases, including pneumonia and scarlet

*Studies and contributions of the Department of Dermatology and Syphilology of the University of Michigan, service of Dr. Udo J. Wile, and from the Bureau of Laboratories, Michigan Department of Health.

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TABLE I

CLASSIFICATION OF 2,490 NONSYPHILITIC CASES IN WHICH THE KAHN TEST AND TWO SEPARATE WASSERMANN TESTS GAVE NEGATIVE REACTIONS

GROUP	DIAGNOSIS	NO. OF CASES
DERMATOLOGY	Acne (various types)	82
	Dermatomycosis	76
	Verruca	71
	Epithelioma	42
	Scabies	39
	Seborrheic dermatitis	35
	Psoriasis	33
	Herpes	29
	Dermatitis venenata	25
	Eczema	24
	Cutaneous tuberculosis	19
	Pityriasis rosea	18
	Furunculosis	18
	Chancroid	16
	Urticaria	16
	Impetigo contagiosa	15
	Erythema multiforme	13
	Scarlet fever	11
	Measles	6
	Varicella	5
	Variola	3
	Miscellaneous	154
	Total	750
MEDICINE	Gastric disease (excluding neoplasm)	69
	Pulmonary tuberculosis	59
	Arthritis	51
	Cardiac disease (nonspecific)	43
	Diabetes mellitus	41
	Hyperthyroidism	33
	Nephritis	28
	Simple adenoma of thyroid	25
	Cholecystitis	19
	Constipation	16
	Bronchitis	12
	Pleurisy	10
	Asthma	9
	Pneumonia	6
	Miscellaneous	74
	Total	495
GYNECOLOGY	Salpingitis	26
	Carcinoma-uterus	17
	Vaginitis	12
	Sterility	14
	Retroversion-uterus	11
	Fibroid-uterus	10
	Prolapse-uterus	8
	Ovarian cyst	6
	Dysmenorrhea	5
	Miscellaneous	42
	Total	151

TABLE I—CONT'D

GROUP	DIAGNOSIS	NO. OF CASES
SURGERY	Gonorrhea and complications	52
	Carcinoma gastrointestinal tract	51
	Injury	48
	Hernia	38
	Appendicitis	37
	Bone and joint tuberculosis	31
	Osteomyelitis	22
	Prostatism	19
	Cholelithiasis	19
	Carcinoma breast	17
	Hemorrhoids	12
	Nephrolithiasis	11
	Carcinoma genitourinary tract	9
	Tuberculosis genitourinary tract	8
	Miscellaneous	124
	Total	498
OTOLOGY	Refractive error	57
	Cataract	41
	Conjunctivitis	23
	Corneal ulcer	9
	Glaucoma	5
	Trachoma	4
	Miscellaneous	44
	Total	183
OPHTHALMOLOGY	Tonsillitis	69
	Otitis media	26
	Sinusitis	25
	Rhinitis	21
	Nasal obstruction	19
	Mastoiditis	14
	Pharyngitis	9
	Tuberculous laryngitis	5
	Miscellaneous	47
	Total	235
NEUROLOGY	Hysteria	29
	Epilepsy	18
	Migraine	11
	Anxiety neurosis	10
	Multiple sclerosis	10
	Tic douloureux	8
	Chorea	7
	Manic depressive insanity	7
	Polyneuritis	6
	Dementia precox	6
	Brain tumor	5
	Paralysis agitans	4
	Miscellaneous	57
	Total	178

TABLE II

CLASSIFICATION OF CASES GIVING POSITIVE REACTIONS WITH ONE OR MORE METHODS

NUMBER OF CASES	KAHN REACTION	WASSERMANN REACTION CHOLESTERINIZED ANTIGEN		DIAGNOSIS
		18 HOUR FIXATION ICE BOX	1 HOUR FIXATION ICE BOX	
1	+++	−	++	Pompholyx
1	++	−	+	Acne rosacea
1	+	−	±	Alopecia areata
1	+	±	±	Acne vulgaris
1	+	−	−	Acne vulgaris
1	+	±	−	Pityriasis rosea
1	++	++	−	Dermatomycosis
1	−	±	−	Pneumonia
1	−	++++	−	Deviated nasal septum
1	−	++++	−	Tonsillitis

fever, and that it gave no false reactions in diabetes, carcinoma and tuberculosis—pathologic conditions in which false Wassermann reactions are still reported, although infrequently.

The several positive reactions obtained are of additional interest. It is perhaps fair to state that the study herein reported extended throughout 1923 and up to July, 1924, and with the exception of two positive reactions (pityriasis rosea and dermatomycosis) all positives were obtained during 1923 before the Kahn test had undergone standardization. These reactions, in our opinion, illustrate that no biologic test can be error proof and emphasize the importance of interpreting the results of serologic tests only in the light of clinical evidence or history.

SUMMARY

A series of 2,500 cases, clinically free from syphilis, were studied with the Kahn precipitation test.

Negative reactions were obtained in 2,493 cases; one plus reactions were obtained in 4 cases; two plus reactions in 2 cases; and a three plus reaction in 1 case.

These results indicate that the Kahn test possesses a high degree of specificity for syphilis.

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HEMOGLOBIN AND IRON IN BLOOD*

By Guy E. Sackett, B.S., M.S., Kansas City, Mo.

THE estimation of hemoglobin is a very unsatisfactory procedure because of the lack of a uniform standard in common for all methods of determining hemoglobin. Most hemoglobinometers are graduated to read in percentage, considering 100 per cent as the normal standard; but yet, each has a different number of grams of hemoglobin which is supposed to correspond to 100 per cent.

Haldane[1] has considered the oxygen capacity of the blood of normal persons to be 18.5 c.c., corresponding to 13.8 grams of hemoglobin per 100 c.c. If the grams of hemoglobin in the blood of normal individuals are determined by the oxygen capacity method or by the iron method and expressed in percentage considering 13.8 grams as 100 per cent, the resulting percentage of hemoglobin will be about 115 per cent, which is, of course, misleading.

Haden[2] working with the Van Slyke oxygen capacity method, found the normal standards to be 15.6 grams of hemoglobin per 100 c.c. of blood.

Table I

Hemoglobin and Iron in Blood of Apparently Normal Men

NAME	RED CELL COUNT	HEMOGLOBIN NEWCOMER METHOD	IRON (FE) MG. PER 100 C.C.	HEMOGLOBIN GM. PER 100 C.C. CALCULATED FROM IRON	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO 100% HEMOGLOBIN BY NEWCOMER METHOD	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO A RED CELL COUNT OF 5 MILLION
W. A. M.	4,896,000	98.5%	55.5	16.56	16.81	16.91
C. W. E.		95.9	53.5	15.97	16.65	
H. M. C.	4,872,000	99.1	55.5	16.56	16.71	16.99
J. D. L.	5,216,000	102.7	57.4	17.13	16.67	16.51
F. L.	4,896,000	98.0	55.5	16.56	16.89	16.91
J. L. H.	5,344,000	101.3	56.0	16.71	16.49	15.63
C. N. C.	4,882,000	99.7	56.4	16.82	16.87	17.22
J. A.	5,216,000	101.3	57.0	17.01	16.79	16.30
J. H. H.	4,832,000	100.0	55.6	16.59	16.59	17.16
H. W.	5,920,000	116.0	63.3	18.89	16.19	15.95
T. J. D.	5,760,000	114.1	64.7	19.30	16.91	16.75
E. M.	4,704,000	94.6	50.2	14.98	15.83	15.92
F. L. S.	4,804,000	95.9	51.5	15.37	16.02	15.99
F. C.	4,800,000	98.4	52.6	15.70	15.95	16.35
D. E. P.	5,160,000	101.0	55.6	16.59	16.42	16.07
Average	5,093,000	101.1	56.02	16.71	16.52	16.47

Average Color Index 0.9925

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From the laboratory of Dr. Myers, Shukert Bldg., Kansas City, Mo.

Newcomer[3] has recently taken 16.92 grams of hemoglobin per 100 c.c. of whole blood, the average obtained by Williamson[4] for adult males, as his standard of 100.

The object of this work is to compare the percentage of hemoglobin found by Newcomer's method with the grams of hemoglobin present, and also to help establish a normal hemoglobin standard expressed in grams per 100 c.c. corresponding to a five million red cell count.

The grams of hemoglobin are calculated from the iron content of the blood. This method used for determining the iron is that of San Yin Wong.[5] To obtain grams of hemoglobin, the number of milligrams of iron per 100 c.c. of blood is divided by 3.35 (since hemoglobin contains 0.335 per cent of iron). The pres-

TABLE II

HEMOGLOBIN AND IRON IN BLOOD OF APPARENTLY NORMAL WOMEN

NAME	RED CELL COUNT	HEMOGLOBIN NEWCOMER METHOD	IRON (FE) MG. PER 100 C.C.	HEMOGLOBIN GM. PER 100 C.C. CALCULATED FROM IRON	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO 100% HEMOGLOBIN BY NEWCOMER METHOD	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO A RED CELL COUNT OF 5 MILLION
P. M.	4,640,000	93.4%	50.8	15.16	16.23	16.33
M. T.	4,800,000	97.0	54.0	16.12	16.62	16.79
B. H.	4,636,000	95.9	51.8	15.46	16.12	16.67
M. A.	4,384,000	88.7	45.7	13.64	15.37	15.55
E. R.	4,800,000	94.6	51.5	15.37	16.24	16.00
F. B.	4 480,000	87.7	47.6	14.21	16.20	15.86
C. E.	5,152,000	100.0	54.6	16.30	16.30	15.82
A. C.	4,288,000	88.7	47.6	14.21	16.02	16.57
J. E.	4,192,000	83.6	45.9	13.70	16.38	16.34
S. C.	4,160,000	84 6	46.3	13.79	16.30	16.57
M. S.	5,312,000	102.7	57.5	17.16	16.71	16.16
K. R.	5,180,000	100.3	55.6	16.59	16.54	16.01
A. S.	4,640,000	96.0	53.7	16.03	16.69	17.27
E. B.	4,800,000	97.1	54.3	16.21	16 69	16.88
Average	4,676,000	93.59	51.20	15.28	16.32	16.34

Average Color Index 1.0008

ence of iron in the chemicals used was ruled out by blank determinations. The blood counting pipette, counting chamber, and cover glass used in this work were calibrated by comparison with apparatus from the U. S. Bureau of Standards.

CONCLUSIONS

1. One hundred per cent hemoglobin by Newcomer's method represents 16.4 grams of hemoglobin per 100 c.c. of whole blood.

2. The normal standard corresponding to a five million red cell count is 16.4 grams of hemoglobin per 100 c.c. of whole blood.

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COMPARATIVE HEAT RESISTANCE OF SERUMS IN THE KAHN AND WASSERMANN TESTS*

By Pearl L. Kendrick, B.S., Lansing, Mich.

IT is generally accepted that prolonged heating of syphilitic serums at 56° C. tends to weaken Wassermann reactions, the explanation being that at this temperature complement-fixing substances are destroyed. According to Noguchi,[1] serums heated for thirty minutes at 56° C. may be 50 per cent less potent than unheated serums. Simon[2] and Kolmer[3] also report the apparent destruction of complement-fixing substances at 56° C. Simon recommends the heating of serums for ten minutes, and Kolmer for fifteen minutes, instead of the usual heating for thirty minutes. In the case of the Kahn precipitation test, it was shown in this laboratory[4] not only that heating serums at 56° C. was essential for precipitation reactions but that heating beyond thirty minutes often gave stronger reactions. This finding suggested a marked difference in the effect of heat on complement fixing and precipitin substances, the former showing apparent destructibility and the latter no destructibility at 56° C.

In the experiments to be reported in this paper an attempt was made to study the comparative effect on the Kahn and Wassermann tests of heating the same serum for various periods. This, it was believed, would give a more correct picture of the relative behavior of the tests with heated serums than experiments in which the Wassermann test was employed with one group of serums and the Kahn test with another group.

EXPERIMENTAL

Syphilitic and nonsyphilitic serums were tested with the Wassermann and Kahn tests before heating and after heating for various periods. The Wassermann test was carried out with a sheep cell—guinea pig complement system. All ingredients entering into the test were used in 0.1 c.c. amounts except the serum which was employed in 0.01 and 0.02 c.c. amounts. Two units of amboceptor, two units of complement and three units of a 0.4 per cent cholesterinized antigen were employed. The fixation period was one hour at ice box temperature. The Kahn precipitation test was carried out in

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the usual manner, employing the standardized routine test. Because of the comparatively large number of examinations made on each specimen, only one tube was employed for each test—the one containing 0.025 c.c. antigen dilution and 0.15 c.c. serum.

Two distinct series of experiments were carried out—the first to determine the effect of heating serums at 56° C. for various periods and the second to determine the effect of heating serums at a higher temperature. In this second series, the temperature chosen was 62° C. since the serum proteins are readily coagulated at still higher temperatures. A large number of the specimens used were diluted with physiologic salt solution to give a sufficient quantity for the several separate examinations. It may be mentioned here that the undiluted specimens showed the same general behavior to heat as the diluted ones and the results of all are, therefore, included together. In each series there was included a group of syphilitic serums and a group of nonsyphilitic serum controls—the classification being based upon the Wassermann and Kahn findings in the routine examinations in this laboratory.

The first series included 66 syphilitic and 36 nonsyphilitic serums. These specimens were examined before heating and after heating periods of thirty, ninety, one hundred and twenty, and one hundred and eighty minutes, respectively, at 56° C. The specimens were prepared for the tests as follows: Two-tenths c.c. was first removed from each specimen before heating. The specimens were then placed in the water-bath at 56° C. for thirty minutes, at the end of which period 0.2 c.c. was removed. The specimens were returned to the water-bath for the next heating period and so on until a portion of each specimen had been removed at the end of each heating period. During the heating periods, the small portions which had been removed were covered with a damp towel to prevent evaporation. When the five portions of each

TABLE I

EFFECT OF HEATING SERUMS AT 56° C. ON WASSERMANN AND KAHN TESTS

SERUM NO.	PERIODS OF HEATING AT 56° C.									
	NONE		30 MIN.		90 MIN.		120 MIN.		180 MIN.	
	WASS.	KAHN.	WASS.	KAHN.	WASS.	KAHN.	WASS.	KAHN.	WASS.	KAHN.
	RESULTS									
1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2	*Ac	++++	++++	++++	++++	++++	++++	++++	++++	++++
3	++	+++	++	+++	±	++++	−	++++	−	++++
4	+++	++	+++	+++	++	++++	++	++++	+	++++
5	++	++	++	+++	+	++++	+	++++	−	++++
6	++++	+	++++	++++	++++	++++	++++	++++	++++	++++
7	++	+	+	+	+	++	±	+++	−	++
8	++++	±	++++	+++	++++	++++	+++	++++	+++	++++
9	++++	±	++	+++	±	++++	±	++++	±	++++
10	+++	±	++	++++	+	++++	++	++++	++	++++
11	++	±	++++	++++	±	++++	−	++++	−	++++
12	±	±	+	++	−	++++	−	++++	−	++++
13	+	±	++++	+++	+++	++++	+++	++++	±	++++
14	Ac	−	+	+	+	++++	+	++++	−	++++
15	++++	−	++++	++++	++++	++++	++	++++	+	++++
16	++++	−	+++	±	++	+	+	++	+	+++
17	++	−	+	+	+	++	±	+++	−	++++
18	++	−	++	+	+	++++	±	++++	−	++++
19	++	−	±	++	−	+++	−	+++	−	++++
20	−	−	+	+	+	++	±	+++	−	+++

*Ac=Anticomplementary.

specimen were ready for testing, they were examined by the Wassermann and Kahn tests. The negative control serums gave negative results throughout. Table I gives the findings with twenty of the positive serums. These results are typical of those given by the whole group.

Before heating, the serums almost invariably gave stronger reactions with the Wassermann test than with the Kahn test. After thirty minutes heating at 56° C., the reactions became stronger with the Kahn test and were comparable with those of the Wassermann. Beyond thirty minutes heating, the reactions with the Wassermann became gradually weaker while the reactions with the Kahn either remained the same or became stronger up to three hours heating. Only an occasional precipitation reaction became weaker even after three hours heating of the serum at 56° C.

The second series of experiments included 101 syphilitic and 21 nonsyphilitic serums. After the routine inactivation period of thirty minutes at 56° C., these specimens were heated for periods of thirty, sixty, and one hundred and twenty minutes, respectively, at 62° C. The several portions of each specimen were removed at the end of the various heating periods as described in the first series and then examined by Wassermann and Kahn tests. The negative serum controls gave negative results throughout. Table II shows the findings with twenty positive serums and, as in the first series, they are typical of the results obtained with the whole group.

TABLE II

EFFECT OF HEATING SERUMS AT 62° C. ON WASSERMANN AND KAHN TESTS

HEATING PERIOD	AT 56° C. FOR 30 MINUTES		AT 62° C. FOLLOWING 30 MINUTES AT 56° C.					
			30 MIN.		60 MIN.		120 MIN.	
SERUM NO.	WASS.	KAHN	WASS.	KAHN	WASS.	KAHN	WASS.	KAHN
	RESULTS							
1	++++	++++	++++	++++	++++	++++	++++	++++
2	++++	++++	++++	++++	++++	++++	*Ac	++++
3	++++	++++	+++	++++	++	++++	++	+++
4	++++	++++	++++	++++	++++	++++	−	++
5	++++	++++	++++	++++	++++	+	−	−
6	++++	++++	+++	++++	+	++++	±	+
7	++++	++++	Ac	+++	Ac	++	Ac	−
8	++++	++++	+	++++	±	++++	−	++
9	++++	++++	+++	++++	Ac	+	−	−
10	++++	++++	−	++++	−	++++	−	±
11	±	++++	−	+	−	−	−	−
12	++++	++++	+	+	−	−	−	−
13	+	+++	−	+++	−	+	−	−
14	++++	+++	±	+++	−	+++	−	++
15	++++	++	++	++	−	+	−	+
16	++	++	−	++	−	++	−	++
17	++++	++	++	++	−	+++	−	+++
18	+	+	−	−	−	−	−	−
19	−	+	−	+	−	±	−	−
20	++	±	++	+++	++	+++	−	++

*Ac=Anticomplementary.

After thirty minutes heating at 62° C., the Wassermann reactions were considerably weaker than after thirty minutes at 56° C. On the other hand, the Kahn reactions showed comparatively little change; a few reactions became somewhat weaker and a few slightly stronger. Beyond thirty minutes heating at 62° C. most of the Wassermann reactions became negative and the Kahn reactions weaker.

CONCLUSIONS

1. Unheated serums give stronger reactions in the Wassermann than in the Kahn test.

2. Serums heated for thirty minutes at 56° C. give comparable reactions in the Kahn and Wassermann tests.

3. After prolonged heating of serums at 56° C.—up to three hours—there is marked weakening of Wassermann reactions with a tendency towards stronger Kahn reactions.

4. Serums heated for thirty minutes at 62° C. show marked reduction in the strength of the Wassermann and comparatively lesser reduction in Kahn reactions.

5. The heating of serums beyond thirty minutes at 62° C. renders practically all Wassermann reactions negative and Kahn reactions weaker.

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THE EFFECT OF SOME GUANIDINE DERIVATIVES AND OTHER RELATED SUBSTANCES UPON THE BLOOD SUGAR OF NORMAL RABBITS*

By Harry E. Dubin, Ph.D., and H. B. Corbitt, Ph.D., New York City

BASED upon the finding that guanidine sulphate produced a hypoglycemia in normal rabbits similar to that resulting after an injection of certain plant extracts, Collip[1] thought that insulin might possibly be a guanidine compound. He found that the hypoglycemia produced by plant extracts is characterized by the fact that low blood sugar does not develop until a number of hours after the injections. The return to normal is considerably delayed. With insulin, on the other hand, hypoglycemia develops in one or two hours and there is a return to normal in from four to six hours. Because of this difference, Collip believed that plant extracts contained a new hormone, which he named "Glucokinin."

Working with crude plant extracts we[2] obtained results exactly like Collip's. We explained the delayed blood-sugar reducing effect as being due to the presence of a blood-sugar increasing substance in crude extracts. When this substance was removed, it was no longer possible to obtain the delayed blood-sugar reducing effect. From the remaining extract, it was possible to isolate a substance which, when injected into normal rabbits, produced a fall in blood sugar exactly similar to that caused by insulin. Obviously therefore,

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we could not support the theory of the presence of a new hormone in plant tissue.

In the case of guanidine sulphate, Collip[3] obtained the delayed blood-sugar decreasing effect, but the animals eventually died. It seemed plausible to assume that insulin might be a guanidine compound and with this in mind, we decided to investigate a number of derivatives and some related substances.

We have studied the effect of guanidine carbonate, methyl guanidine sulphate, guanidine nitrate, guanidine hydrochloride and guanidine thiocyanate. In addition, we tested histidine, present in germinating plants; xanthine, found in cellular tissue; and adenine, present in the pancreas. The experimental procedure has already been described.[2]

A study of the tables shows that histidine hydrochloride was without effect. Adenine sulphate caused a 10.4 per cent decrease in six hours, while xanthine produced a fall of 23.2 per cent in the same length of time. Observations for long periods of time, with these materials, were not made.

As for the various guanidine derivatives, the most pronounced hyperglycemia was obtained with methyl guanidine sulphate. The thiocyanate in one case caused a rise of 45.8 per cent in six hours. In another rabbit, there was an increase of 124.0 per cent in five and two-thirds hours. The animal died after twenty-five hours, the blood sugar at that time being 15.1 per cent below the original reading. This decrease is frequently observed as well in normal animals at death.

Of all the substances investigated, it may be said in general that small doses were ineffective. Larger doses did cause a reduction in blood sugar, but the animals eventually died. Our findings in this respect, therefore, confirm those of Collip.

At the same time, it should be noted that the decrease in blood sugar

TABLE I

BLOOD SUGAR OF NORMAL RABBITS AFTER INJECTION OF VARIOUS GUANIDINE DERIVATIVES

RABBIT NO.	RABBIT WT.	PREPARATION INJECTED	DOSE	TIME AFTER INJECTION	BLOOD SUGAR	CHANGE IN SUGAR CONTENT	REMARKS
	GM.		MG. PER KG.	HR.	MG. PER 100 C.C.	PER CENT	
66	2105	Guanidine carbonate	10	0	112		
				2	115		
				4	111		
				6	111		
51	1570	Guanidine carbonate	50	0	135	+17.0	
				2	141		
				4	147		
				6	158		
55	1580	Guanidine carbonate	150	0	148		
				2	142		
				4	135		
				6	103	−30.4	
				23½	114		
				29	140		
56	1710	Guanidine carbonate	200	0	145		
				2	124		
				4	93		
				6	85	−43.4	Died at about 50 hours.
				24	98		
				29	85		

TABLE II

BLOOD SUGAR OF NORMAL RABBITS AFTER INJECTION OF VARIOUS GUANIDINE DERIVATIVES

RABBIT NO.	RABBIT WT.	PREPARATION INJECTED	DOSE	TIME AFTER INJECTION	BLOOD SUGAR	CHANGE IN SUGAR CONTENT	REMARKS
	GM.		MG. PER KG.	HR.	MG. PER 100 C.C.	PER CENT	
49	920	Methyl guanidine sulphate	50	0	99		
				2¼	91		
				4½	86		Animal died 25 hr.
				6⅓	85	−14.1	
68	1245	Methyl guanidine sulphate	100	0	95		
				2½	56	−41.0	
				3	—		Dead; bled at once
				3	25		
59	1800	Guanidine nitrate	200	0	89	−73.7	
				2	61		
				4	62		
				6	57		Died during night
63	2150	Guanidine hydrochloride	180	0	138	−36.0	
				2½	129		
				4	79		
				25	110	−42.7	
62	2245	Guanidine thiocyanate	180	0	126		
				2⅓	196		Died at 25 hr., bled at once
				5⅔	283		
				25	107	+124.0	

TABLE III

BLOOD SUGAR OF NORMAL RABBBITS AFTER INJECTION OF VARIOUS PURE COMPOUNDS

RABBIT NO.	RABBIT WT. GM.	COMPOUND INJECTED	DOSE MG. PER KG.	TIME AFTER INJECTION HR.	BLOOD SUGAR MG. PER 100 C.C.	CHANGE IN SUGAR CONTENT PER CENT	REMARKS
46	3070	Histidine hydrochloride	100	0	104		
				2	107		
				4	102		
				6	116		
			500	0	119		
				2¼	117		
39	1610	Adenine sulphate	100	3½			
				4½	131		
				5½	124		
44	1365	Adenine sulphate	325	0	106		
				2	174	+64.2	
				4	187	+76.3	
				6	95	−10.4	
48	1740	Xanthine	56	0	95		
				2	88	− 7.4	Other animals gave similar results.
				4	84	−11.6	
				6	73	−23.2	

was not comparable, in any instance, with that produced by a unit of insulin. Accordingly, we feel that there is no relationship between insulin and any of the products tested.

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THE VALUE OF EARLY WASSERMANN EXAMINATIONS IN THE DIAGNOSIS OF VENEREAL LESIONS*

By Robert G. Owen, A.M., M.D., and H. E. Cope, B.S., M.D., Detroit, Michigan

IN May of 1923, this laboratory adopted the following routine for the diagnosis of suspected primary syphilitic lesions. At first examination, we obtain blood for a Wassermann test, in addition to the usual dark-field slides. Cases with negative dark-field and Wassermann examinations are recalled for future Wassermann examinations at stated intervals, to cover a period of six weeks from the onset of their lesion; i. e., the time within which a positive Wassermann usually develops.

In a survey of 1160 dark-field examinations we were astonished by the relatively large number of first examinations yielding negative dark-fields and positive Wassermanns. The consensus of opinion among those engaged in clinical laboratory work seems to be that a Wassermann is of little value if taken early in the course of a venereal sore, the onset of a positive Wassermann being generally recognized as occurring between the fourth and eighth week. In those cases in which we have followed the development of positivity, it has occurred quite uniformly between the twenty-first and thirty-sixth day from the appearance of the initial lesion.

In spite of the unreliability of the average venereal history, one cannot but be impressed when 2 per cent of all patients with venereal lesions show positive Wassermanns within fourteen days of the onset of the sore, and before the development of a typical luetic inguinal adenitis. After discarding all female cases and all in which the sore has been present over fourteen days, we found twenty-two with negative dark-field examinations and positive Wassermanns. The obvious interpretation to be placed on these findings is that of previous infection, which is borne out by the fact that we obtained a history of previous infection from eleven of these twenty-two cases.

The purpose of this note is to emphasize that people with syphilis may still contract venereal sores and that with negative dark-field examinations and delay in making a Wassermann test until the usual time for beginning positivity, a certain percentage will finally be labeled as chancres which are in reality chancroids superimposed upon a previous latent syphilitic infection. We, therefore, wish to call attention to the value of early Wassermanns and to urge the adoption of such examinations by those engaged in this work.

*From Serological Department, Owen Clinical Laboratory.
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LABORATORY METHODS

AN HEMATOCRIT METHOD*

By C. M. Van Allen, M.D., New York

THE hematocrit is an instrument by which is determined the volumetric relationship between corpuscles and plasma in a specimen of blood. Separation of the two elements is brought about in the centrifuge by virtue of a difference in their specific gravities.

Since the invention of the hematocrit, in 1891, by Hedin,[1] a great many types and modifications have appeared. They may be classified for the purpose of description into two groups, depending upon whether minute amounts of blood are employed in a capillary tube, or larger amounts (5 to 15 c.c.) in a centrifuge tube. Some of the methods use whole blood and aim to complete sedimentation before coagulation takes place, and others inhibit coagulation to begin with by mixing the specimen with an anticoagulant.

The original hematocrit of Hedin is a capillary tube and has remained, with minor modifications, the most widely used of that type. It consists simply of a finely graduated glass tube about 10 cm. in length. The blood is collected in a separate container and mixed with a known proportion of isotonic anticoagulant solution (sodium sulphate 1 part; potassium bichromate 2 parts; water 100 parts), and a sample of this mixture is drawn up into the capillary tube. The ends of the tube are closed securely by fastening it in a specially constructed centrifuge head. Centrifugalization is then carried on at the rate of 10,000 rev. per min. for fifteen minutes or so, sedimenting the cellular content into a solid column at the outer end of the hematocrit tube. The height of this column in relation to the height of the total column occupied by the specimen represents the volumetric relationship of the corpuscles in the mixture examined. This ratio is then corrected for the amount of diluent added, to obtain the value for whole blood.

The other type of hematocrit is the more popular at the present time, chiefly because it can be homemade and is used with the ordinary power centrifuge. It consists of a graduated centrifuge tube. Ten or fifteen cubic centimeters of blood are drawn by venepuncture into a syringe and deposited in the tube with a quantity of anticoagulant, dry or in solution. After centrifugalization at high speed, the corpuscular sediment is measured upon the scale and corrected, if necessary, for dilution.

There is little purpose in entering upon description of the various modifications of these two types of hematocrit that have appeared, although many are very ingenious. None of them has gained any considerable popularity,

*From the Laboratories of The Rockefeller Institute for Medical Research, New York. Received for publication, December 23, 1924.

and in each case the reason for this is to be found in a deficiency from one or both of two standpoints—practicability of operation and accuracy. Whole blood is not at all easy to handle and measure with precision. Its viscidity renders it somewhat adherent to the walls of a containing vessel, and difficulties readily arise in transferring a specimen accurately and cleanly from the collecting instrument (syringe or pipette) to the hematocrit tube. If the syringe is used for the purpose, the "dead space" in the extremity also complicates the technic. The slightest coagulation taking place in the specimen prevents complete sedimentation of the corpuscles, and in normal blood coagulation is initiated far sooner after shedding than the usual methods for estimation of clotting time would indicate. For instance, the wire loop method of Inchley[2] gives the clotting time of cat's blood as between 100 and 200 seconds, and by a wire loop method of my own[3] it is possible to detect coagulation in normal rabbit's blood as early as between twenty and thirty seconds after shedding. Apparently the Boggs and other commonly used procedures measure, not the initiation of clot formation, but the attainment of a certain degree of firmness or resistance in the clot, and subsequently their readings are taken well along in the process of coagulation. On account of interference by coagulation, the employment of whole and unaltered blood in the hematocrit necessitates having the centrifuge directly at hand, often an impracticable specification with the high-power machine. There is a distinct disadvantage, also, in those hematocrits which require larger specimens of blood than can be obtained readily by needle prick of the finger or ear lobe.

The physical principles underlying the operation of the hematocrit are well understood; nevertheless, many hematocrit methods have not in all respects held strictly to these principles. The most frequent inconsistency has to do with the use of an anticoagulant with the specimen of blood. The majority of investigators who employ an anticoagulant solution select the strength of the solution on a purely arbitrary basis. A very slight alteration of the osmotic pressure of the fluid medium surrounding red blood corpuscles results in a perceptible change in their volume, and care must be taken that the concentration of a diluent used in hematocrit determinations be such as to render it isotonic with the blood plasma. A common error arises also in the supposition that this difficulty may be overcome by the use of the anticoagulant in powdered form. Hooper, Smith, Belt, and Whipple[4] state that the addition to whole blood of the minimum amount (0.1 per cent) of sodium oxalate crystal that will completely inhibit coagulation changes the corpuscle volume by 3 per cent. Our own observations indicate that the addition of such an amount of the crystal to whole blood changes the corpuscle volume by 5.5 per cent. Most of those using a dry anticoagulant employ more than the minimum amount.

Sufficient detail in regard to the manner of centrifugalization is seldom given, and this is an important omission, because up to a certain point the degree of sedimentation is roughly proportional to the total centrifugal force exerted upon the corpuscles, and this force is a function involving several factors. In order to be sure of obtaining complete separation of the cor-

puscles from the fluid element, it is not sufficient to give as specifications for centrifugalization only the velocity and duration. The packing power that is obtained from one centrifuge can be duplicated in another only when, in addition to velocity and duration, are known the radius of the centrifuge head, the diameter of the hematocrit lumen and the dilution of the specimen. As will be demonstrated below, all of these factors play a perceptible rôle. Koeppe[5] has shown that, coincident with the attainment of an ultimate reading in the hematocrit, the red corpuscle mass assumes a peculiar translucency to transmitted light and at the same time an opacity to reflected light, and he suggests that this appearance is the result of the elimination of all interstitial fluid.

Very probably it is largely because of the complications of technic and the factors of error mentioned above, that the hematocrit has been very largely discarded from clinical use in favor of the hemacytometer, for certainly the information to be gained from the former instrument is of no less value than that rendered by the latter. Indeed, it would appear from what we know of the alterations in the size of the average erythrocyte that take place in the anemias, that volume determinations must represent erythrocyte function in pathologic conditions more truly than do cell counts. It is well recognized, for instance, that the physical vigor of a patient suffering from pernicious anemia and with one-quarter the normal number of red cells is considerably greater than that of a patient with a secondary anemia of like cell count. This is readily accounted for by the changes in erythrocyte size characteristic of these two diseases, for the blood of the first patient, when tested from the volumetric standpoint may easily be found to possess three-eighths of the normal amount of erythrocytic substance, and the blood of the second patient to contain only one-sixth. This advantage of the volumetric over the numerical estimation has been made of use in the prognosis of tuberculosis[6] and of other constitutional diseases.

However, there should be, of course, no competition between the hemacytometer and the hematocrit, for they measure quite different characteristics of the blood, each in itself of importance in understanding the status of the hemic system. This is brought out strikingly in the "volume index" of Capps,[7] which is the ratio between volume and numbers of red corpuscles in a specimen of blood. Haden[8] has recently emphasized the value of the volume index in the diagnosis of pernicious anemia. It is apparently more to be trusted than the color index. He asserts that a high volume index in association with achlorhydria is virtually pathognomonic of the disease even at a very early stage. This in itself is sufficient justification for including the hematocrit in the equipment of the clinical laboratory.

It has been necessary in some of our work to determine the relative corpuscle volume of rabbit's blood from small specimens and with considerable accuracy. A hematocrit method has been contrived which has given complete satisfaction from these standpoints under very extensive use, and which at the same time is simple of operation. No claim is made for originality in any single detail of the instrument or the method, for these are to be found in various of the hematocrits hitherto described, but so far

as can be found there is no hematocrit which combines these features or a technic which meets the requirements of simplicity and accuracy as adequately as does this. Because of the apparent need of a hematocrit of these characteristics in experimental and clinical work, the following description is given of the instrument and the method of its use together with sufficient study of the technical factors involved to insure reliable performance.

DESCRIPTION OF INSTRUMENT AND METHOD OF USE

The hematocrit is of the capillary type and is operated with either whole or altered blood. Sedimentation is performed in a power centrifuge.

Instrument.—Drawings of the instrument to be described are given in Chart 1, representing it ready for the collection of the blood specimen and, again, ready for centrifugalization.

The hematocrit is a glass pipette with a chamber, somewhat similar in design to the hemacytometer pipette. It is 10 cm. in length over all, of which the shaft occupies 7 cm. The bore of the shaft is about 0.5 mm. in diameter and should be perfectly uniform throughout. The shaft ends bluntly at its lower extremity and is graduated from the tip up to within a short distance of the chamber in a scale of 100 parts. The chamber is pear-shaped, larger end up, and has a capacity of about 1 c.c. The short length of tubing at the upper extremity of the pipette is also blunt tipped and serves for the attachment of the suction tube. The pipette is equipped with a rubber mouth suction tube. A rubber band, 7 to 9 cm. in length and 1 cm. wide, is employed for the purpose of sealing the ends of the pipette after collection of the specimen.

Method.—The hematocrit pipette is first prepared for use by attaching the suction tube. A free flow of blood upon the skin surface of the ear lobe or finger tip is obtained by needle prick and the first drop or two are discarded. As a fresh quantity appears it is drawn up into the bore of the pipette shaft to the top of the scale. Blood that adheres to the outside of the pipette is now quickly wiped away and, turning to a stock 1.3 per cent sodium oxalate solution, a small quantity of this anticoagulant is drawn with the specimen into the chamber of the pipette, about half filling it. Loss of fluid from the pipette is guarded against by placing a finger over the lower end of the tube bore, while the suction tube is removed and the pipette is enclosed from top to bottom with a rubber band, as illustrated in the drawing. No shaking or mixing of the specimen is necessary other than that which occurs inadvertently.

The pipette thus adjusted is placed in the centrifuge cup, shaft end down and properly counterbalanced, and centrifugalization is carried on at 2,700 rev. per min. or at any convenient rate above this for about fifteen minutes. Removing the pipette from the centrifuge at the end of this time, the volume reading of the red cell content is made directly, in percentage, from the shaft scale by noting the upper limit of the mass of red sediment. No correction for dilution is needed.

Provided the collection of the blood specimen has been accomplished rapidly enough to prevent clotting—and this is not at all difficult with a

little practice—the upper limit of the column of red cells to be found at the end of centrifugalization is sharply defined, and directly over it rests a thin layer of white substance. This layer is that commonly referred to as the "buffy coat," and it may be included with the red column in making the corpuscle reading, or not, as desired.

In place of sodium oxalate as an anticoagulant, powdered hirudin or powdered heparin may be employed. We have had no experience with these agents but suggest that the following procedure be used: A few particles of the powder are placed upon the skin at the spot chosen for puncture. Mixture of the agent with the specimen takes place automatically as the blood wells up from the wound. A measured portion of this altered blood is secured in the hematocrit as described above, but the blood is allowed to remain without dilution in the shaft bore. The pipette is enclosed in the rubber band and centrifugalized. Rotation at 2,700 rev. per min. for one hour should be sufficient to obtain a constant reading.

Precisely the same technic as the foregoing may be employed without any form of anticoagulant. Considerable haste is necessary in the procedure, however, in order that the corpuscles may be separated from the plasma before clotting begins, and for this purpose the centrifuge must be directly at hand and all adjustments anticipated. This method probably gives the most reliable readings of corpuscle volume as there has been nothing introduced to disturb the osmotic pressure of the blood plasma, but it is not practicable for most purposes, particularly for clinical use.

Precautions in Technic.—Certain precautions should be emphasized in regard to the collection of the blood specimen. When drawing the blood into the pipette bore any considerable overflow past the mark should be avoided, for in reducing the level of the blood to the scale an appreciable quantity may remain adherent to the glass and give rise to too high a final reading. The best procedure is to allow the blood to enter to a point slightly above the mark and then, in wiping off the outside of the pipette with a gauze sponge, the tube bore may be touched lightly and the height of the blood column adjusted to the required mark. When the specimen has been accurately measured in this way, it is advisable immediately to draw the specimen on up the shaft a short distance so that when the tip of the shaft is immersed in the diluting solution none of the blood will be accidentally lost. This maneuver is feasible with this instrument because accuracy of dilution is not striven for. After the specimen is diluted with the anticoagulant solution there need be no further haste in the procedure.

The use of an ordinary rubber band to seal the ends of the pipette during centrifugalization has been found entirely satisfactory. The method is simple enough and there has never been evidence of leakage or other source of error attributable to it in routine use. There may be difficulty, however, from perforation of the band by the tube if the lower extremity of the pipette shaft is not fairly flat and smooth, or if centrifugalization is carried on at very high rates of speed.

RESULTS

Our use of the hematocrit has been confined to rabbits, and several hundred determinations have been made upon these animals, both normal and diseased. The data from 40 normal rabbits, picked at random from this material, are summarized in Table I.

Normal hematocrit values for the human being as given by Gram and Norgaard[9] are probably the most accurate available. The investigators used hirudin as an anticoagulant and a modified Hedin hematocrit. Sedimentation was accomplished at 3,000 rev. per min. for one and one-half hours. A summary of their data is given in Table I.

In the rabbit, under conditions of anemia, the relative red cell volume may be reduced to 18 per cent, and in man to 5 per cent.[8]

TABLE I

SPECIES		RABBIT	HUMAN				
Sex		Male	Male	Female	Both Sexes	Both Sexes	Both Sexes
No. of Subjects		40	10	10	20	20	10
Relative Red Cell Volume	Maximum	54%	50%	43%	50%	51%	52%
	Minimum	33%	42%	39%	39%	37%	41½%
	Mean	39%	46%	41%	43½%	44½%	48½%
Method		Author's with 1.3% sod. oxalate	Gram and Norgaard with hirudin			Gram with 3% sod. citrate	Haden by method of Hooper *et al.* 1.6% sod. oxalate

EXPERIMENTAL STUDY OF UNDERLYING PRINCIPLES

Attention has been called, in the introduction of this paper, to certain important physical principles underlying the operation of the hematocrit. These principles have to do with the conservation of the natural size of the red corpuscles in the specimen of blood and with the attainment of complete sedimentation. Both objects are equally necessary in order that the readings obtained with the instrument shall truly represent the relative corpuscle volume as it exists in the circulating blood of the subject. The first object requires that the anticoagulant used shall not alter the osmotic pressure of the blood appreciably. If the anticoagulant is used in solution its concentration must be such as to be isotonic with the blood plasma, and if the agent is used in powdered form the amount added necessary to inhibit coagulation must be so small as not perceptibly to raise the osmotic pressure of the plasma. The second object requires that the centrifugal force employed to obtain sedimentation shall be sufficient to reduce the body of sediment to its ultimate size. The effect of centrifugal force in this instance is determined by several factors, among which are the speed, duration and radius of rotation, the distance through which the cells have to be driven, and the resistance offered to their passage.

The influence of each of these principles in the operation of the hematocrit has been studied experimentally, and upon the information thus afforded

have been based the specifications for the method of use of the instrument as given. The experiments are described herewith.

Strength of Anticoagulant.—Sodium oxalate solution was selected as the anticoagulant in our work. We have not been able to find accurate physicochemical investigations determining the osmotic pressure of sodium oxalate solutions relative to that of the blood plasma. Hooper, Smith, Belt, and Whipple were likewise unable to obtain information on this point and solved the problem for themselves by using red corpuscle volume as an indicator of isotonicity between the two fluids in the following manner: Hematocrit determinations (syringe method described above) were made upon several sam-

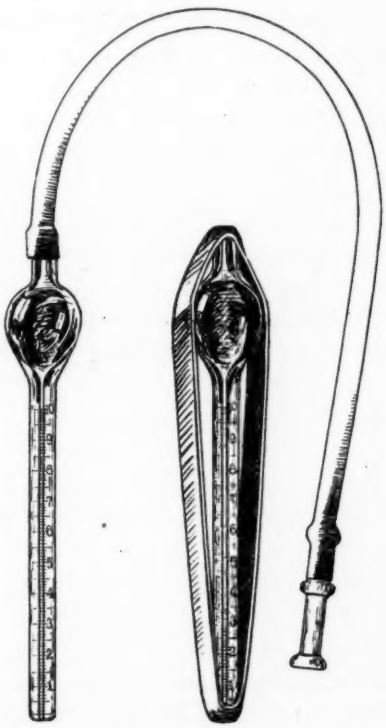

Chart 1.—The hematocrit pipette, equipped with mouth suction tube and sealed with rubber band ready for centrifugalization.

ples of blood from the same animal (dog), using varying strengths of sodium oxalate solution as diluents. A determination of cell volume was also made upon a sample of the same blood, defibrinated and without dilution, as a control expressing the true relative corpuscle volume; and by comparing this value with the other readings it was possible to select the particular solution of sodium oxalate rendering a correct volume estimation. Two separate experiments of this sort agree exactly upon the required strength of sodium oxalate as 1.6 per cent. The data of one of these are reproduced in Table II, together with the results of another experiment which was performed as a check upon the work. The method of this third experiment was the same as of the others, except that, instead of using defibrinated blood as a control, the noncoagulable blood of a dog given a peptone injection was employed.

The data obtained were not full enough to determine an exact result but a very close agreement is indicated with the other experiments. These investigators, accordingly, adopted 1.6 per cent sodium oxalate solution in their work with the hematocrit.

While the agreement between the results obtained in the above experiments suggests the reliability of defibrinated and "peptone shock" bloods as controls of normal corpuscle volume, yet certain objections to these methods are justifiable. As regards defibrination, it would appear that in removing the fibrin from the blood a portion of the corpuscles is necessarily lost; and in "peptone shock" Descamps[10] has shown that the average size of the

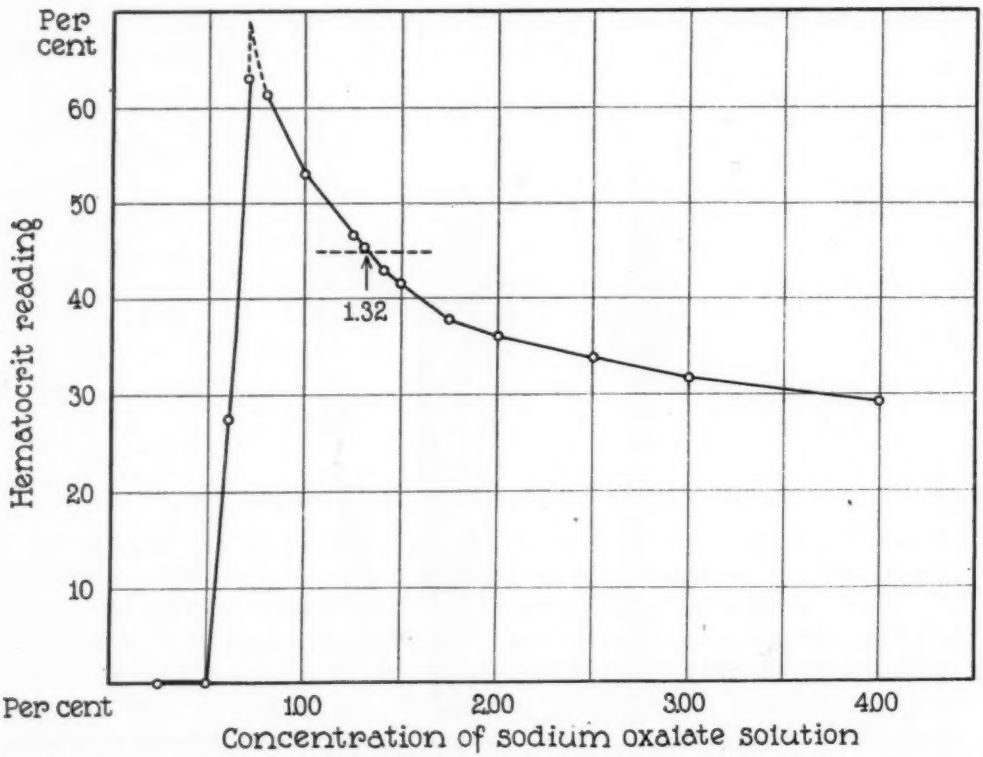

Chart 2.—Hematocrit reading of unaltered blood as compared to readings obtained upon the same blood treated with various concentrations of sodium oxalate in water.

erythrocytes is reduced. Accordingly, both types of blood used as controls for corpuscle volume would give values in terms of oxalate solution somewhat too high. However this may be, it is simpler to use for this purpose determinations made upon whole and unaltered blood.

We have repeated these experiments upon rabbit's blood, using the capillary hematocrit here described and unaltered blood as control. A series of readings were made upon the blood of a rabbit with sodium oxalate solutions of varying concentrations and, to compare with them, a "dry" reading upon the same blood (see technic given above).

The data of one of these experiments are represented graphically in Chart 2. The volume readings obtained with the various solutions are plotted

upon the chart and the points connected by a continuous line. The curve thus formed assumes a characteristic shape. Commencing with a volume of 0 per cent at 0.50 (per cent sodium oxalate), the curve rises sharply to 63 per cent at 0.70, and then begins to fall more and more nearly approaching the horizontal. The last reading, 29.2 per cent, was obtained from a 4 per cent solution. The reading of the hematocrit from unaltered blood ("dry" reading) is added to the chart as a horizontal dotted line at 45 per cent volume, and this line intersects the curve at a point corresponding to 1.32 per cent sodium oxalate.

Three other experiments of this sort have resulted by indicating 1.28, 1.32 and 1.44 per cent solutions of sodium oxalate as isotonic with the plasma of different normal rabbits. This variation in readings is probably not greater than the natural variation in osmotic pressures of the blood plasma of different normal individuals, and it is such that, should 1.30 per cent oxalate solution be employed routinely, the maximum deviation from the mean that would occur in the results on this account would be 1.25 per cent. In disease conditions with alteration of the osmotic pressure of the plasma beyond the normal range, there would arise greater error than this, but probably not so great as to exceed an allowable degree of inaccuracy. Should it be desirable at any time, because of osmotic pressure changes, to obtain more accurate results, the substitution of the "dry" in place of the "wet" method is to be relied upon. The degree of accuracy of this instrument, as indicated by these figures, is apparently considerably greater than that of the hemacytometer. In the latter instrument there enters the factor of personal equation to a considerable extent, which accounts largely for the variation often obtained between duplicate counts, even in skilled hands. Gulland and Goodall[11] place the maximum deviation from the mean reading with the instrument at about 11 per cent.

Force of Centrifugalization.—The centrifugal force exerted upon the cells in the hematocrit has been studied from the standpoint of its chief contributory factors, with the purpose of ascertaining the effect each exerts upon the process of sedimentation. From this information it should be possible to determine the conditions of centrifugalization which obtain complete sedimentation most economically and conveniently. Experiments are here described, consisting in hematocrit determinations upon rabbit's blood performed in the routine way with 1.3 per cent sodium oxalate solution, under conditions in which first one and then another of the factors affecting the centrifugal force is made to vary. The data are represented graphically in Chart 3.

Duration of Centrifugalization.—Three separate hematocrit determinations were made upon specimens of blood taken from the same rabbit by the routine method, centrifugalized at 2,700 rev. per min. Each specimen was read after three minutes of centrifugalization, after five minutes, after seven minutes, after eleven, fifteen, and twenty minutes, and these values were plotted to form curves representing the progress of the concentration of the corpuscle sediment.

These curves are reproduced in Chart 3 at B. It will be seen that they

TABLE II

STRENGTH OF SODIUM OXALATE	VOLUME READINGS EXPERIMENT 1	VOLUME READINGS EXPERIMENT 3
1.0	48.0	63.7
1.1	47.7	
1.2	47.4	
1.3	46.6	
1.4	46.1	61.1
1.5	46.3	
1.6	45.2	
1.7		56.6
	Defibrinated blood control	"Peptone shock" control
	45.1	59.6

behave in a fairly uniform fashion, descending together from left to right at a decreasing rate until at the fifteen minute point a level is reached that remains nearly constant. One may infer from this, that under the circumstances of the test as routinely performed centrifugalization for fifteen minutes is necessary in order to obtain a constant reading. The final values found in these three determinations were 33.0, 33.5, and 33.5 per cent respectively. This, by the way, is the usual degree of variation obtained in duplicate readings with this instrument.

Speed of Centrifugalization.—The preceding experiment was repeated upon another rabbit precisely in the same way, except that one specimen was centrifugalized at 2,000 rev. per min., another at 2,700 rev. per min., and the third at 3,600 rev. per min. The data secured were plotted as before.

The curves obtained are reproduced in Chart 3 at A. They conform to the type of those at B, but differ as regards the slope of descent. That is, at a speed of 2,000 rev. per min. (Curve 1) a constant value was reached after twenty minutes, at 2,700 rev. per min. (Curve 2) a constant value was obtained at fifteen minutes, and at 3,600 rev. per min. (Curve 3) the volume reading became constant from the eleventh minute on. There is a difference in the curves, also, as regards the final readings, for, while the levels of concentration reached by the sediment at the two higher rates of speed agree perfectly, at 2,000 rev. per min. the final reading of the corpuscles fell considerably short of the others.

It is to be inferred from this, that under the routine conditions of the test the rapidity of sedimentation increases with the rate of centrifugalization, and that the ultimate concentration of the sediment is also in proportion to the rate of centrifugalization up to a certain point (2,700 rev. per min.), above which rate there is no further change in the final reading.

Hematocrit Bore and Specimen Dilution.—In these experiments the hematocrit method differed from the routine procedure only as regards the diameter of the hematocrit bore and the degree of dilution of the specimen. An hematocrit was used, the shaft bore of which measured 4 mm. in diameter. Two determinations were made upon a rabbit; in one the specimen of blood was diluted by the anticoagulant to a degree approximately equal to that ordinarily employed in the capillary hematocrit, and in the other the dilution was at least five times as great (a degree impossible with the capillary instru-

ment). Readings of the two specimens were taken at various stages during centrifugalization as in the other experiments.

The curves constructed from the readings in these experiments are given at C in Chart 3. Curve No. 1 is from the highly diluted specimen; it reaches a level at fifteen minutes. Curve No. 2 is from the normally diluted specimen, and it reaches a level at five minutes. It is to be pointed out that the conditions underlying the determination of Curve No. 2 are identical in all respects with those of the curves at B except for the diameter of the bore of the hematocrit used.

It would appear, accordingly, that with other factors constant the rapidity of sedimentation is delayed by excessive dilution of the specimen of blood and accelerated by an increase in the diameter of the hematocrit bore, but that these effects are not to be expected as a result of the minor variations that might take place in the technic of the capillary hematocrit.

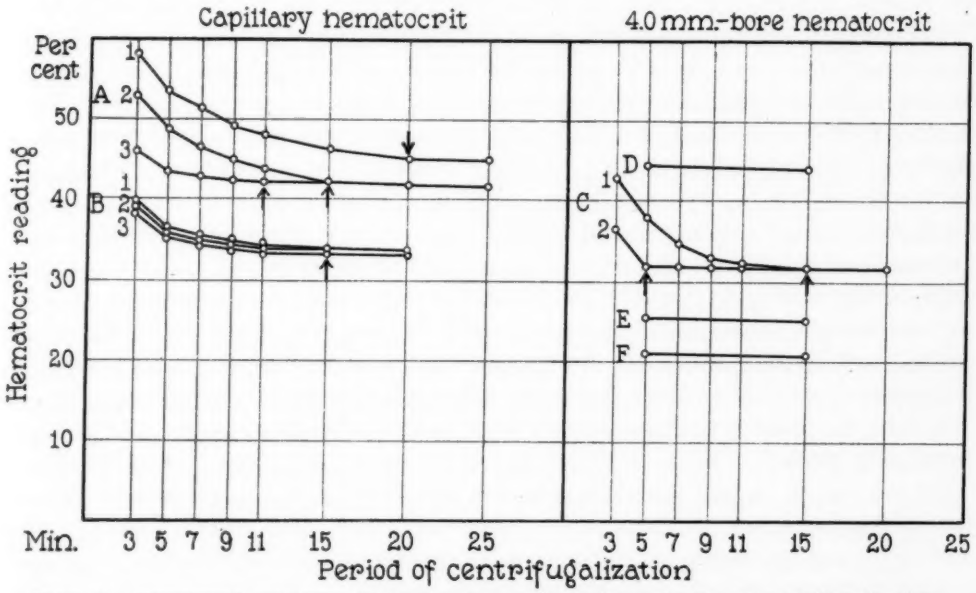

Chart 3.—Hematocrit readings obtained under various circumstances of centrifugalization.

Degree of Anemia.—The 4 mm.-bore hematocrit has been used for relative corpuscle volume determinations on rabbits in various stages of secondary anemia, and in some of these determinations readings were taken at five minutes as well as after the customary period of fifteen minutes of centrifugalizaton, for the purpose of testing the process of sedimentation in blood specimens of widely different corpuscle content. The same technic was employed as that used in the experiment producing Curve 2 at C. Three of these determinations are represented graphically in Chart 3 at D, E, and F. By comparing them with Curve 2 at C, it is readily to be seen that in various grades of anemia the process of sedimentation of the corpuscles in the hematocrit is not different from that which occurs in normal blood.

Radius of Rotation.—The question of the effect of variation in the radius of the centrifuge head upon the process of sedimentation of blood corpuscles

has not been approached experimentally. It is, of course, to be expected that variation in that factor would bring about changes in the centrifugal force. Since it has already been found that, provided the centrifugal force be kept above a certain amount and be allowed to act sufficiently long, an ultimate and constant volume of the corpsucles is reached, this factor may be disposed of, along with the questions of dilution and of tube bore, by laying down the following specifications for centrifugalization with the author's capillary hematocrit:

Centrifugalization of the specimen should be carried on at a rate of at least 2,700 rev. per min. for about fifteen minutes; and this without regard for the radius of the centrifuge head, providing it is greater than 9 cm., or for the slight variations that may occur in the diameter of the hematocrit bore and in the degree of dilution of the specimen.

DISCUSSION

In standardizing a hematocrit method for use in our work, as has been described, effort was made to procure a considerable degree of accuracy without greatly complicating the technic. The almost exact agreement that occurs between duplicate readings with the instrument has created confidence in its use in our hands as to uniformity of behavior, and this apparently is to be accounted for by the fact that the specimen is collected and measured under the same scale as is used for the final reading, eliminating entirely the necessity of a separate collecting instrument. Test of the physical principles underlying the operation of the hematocrit has assured a reasonable degree of conformity between its readings of cell volume and the actual volumetric status of the corpuscles in the circulating blood. The method should be suited to the various circumstances of the experimental or clinical laboratory. The pipette itself is the only special apparatus necessary; a convenient period of time is allowable between the collection of the blood and centrifugalization; and the requirements for sedimentation can be met by the standard power centrifuge.

There is one feature of the method that may need readjustment to new circumstances. The strength of the anticoagulant that we have used, 1.3 per cent sodium oxalate solution, is in agreement with the conditions of osmotic pressure of rabbit's blood, but it may not be the ideal concentration for use with the blood of another species. Before the test is employed upon the human being, for instance, it would be desirable to reinvestigate the question of the isotonicity of the anticoagulant solution, and this could be done simply by repeating the experiment depicted in Chart 2, it being necessary to employ only those strengths of the solution ranging within the zone of probable isotonicity with the blood. Readjustment of the anticoagulant for the species of animal would, indeed, be necessary in exacting work with the hematocrit, such as in determining total blood volume, but in the routine use of the instrument for the estimation of anemia, comparative values only are required, and here the use of the 1.3 per cent solution should be satisfactory. There would probably be some slight error in the readings obtained, but, whatever the error, its degree would be constant throughout. Those using the hematocrit

of Hooper, Smith, Belt, and Whipple for testing human blood have employed the strength of sodium oxalate which the authors had found suited to dog's blood, apparently without investigating its adaptability.

It would not be permissible to employ with this instrument a solution that has been shown to render correct readings with another hematocrit method. This follows from the fact that the adjustment of the concentration of an anticoagulant to a particular method means adjustment to secure accurate values of corpuscle volume in spite of the inconsistencies that the method may possess, such as the "dead space" of the collecting syringe, the disparity between the scale of graduations of the collecting instrument and that of the hematocrit, and incomplete sedimentation of the corpuscles. We have found, for example, that the syringe hematocrit technic (Hooper *et al.*) requires a 1.5 per cent solution of sodium oxalate when used for rabbit's blood, and that with this strength of solution it renders values identical with those obtained with 1.3 per cent sodium oxalate and the capillary hematocrit. Probably neither of these concentrations of the salt is actually isotonic with the blood of the rabbit.

In describing the experiments performed for the standardization of the present hematocrit method, and in drawing conclusions from them, it was stated that, after fifteen minutes of centrifugalization at 2,700 rev. per min. with a centrifuge head of 9 cm. radius, a constant volume of the sediment is attained. This is not strictly so. Greatly prolonged centrifugalization gradually reduces the corpuscle volume still further, and in order to obtain an absolute volume the process has to be carried on for an hour or more. This fact is responsible for the long periods of centrifugalization advocated by various workers, and it is for this reason, too, that we advise prolonged centrifugalization when the hematocrit is used without an anticoagulant or with an anticoagulant in powdered form. The inconvenience of such a procedure can be done away with, however, by adjustment of the diluting solution to such a strength that the volume of sedimented corpuscles obtained after a conveniently short period of centrifugalization, although not absolute for that particular specimen, is identical with the actual corpuscle volume as present in the circulating blood, by virtue of the fact that the surrounding fluid is somewhat hypertonic. We have selected a fifteen minute period, because the relative constancy of the sediment volume from this point on allows for a margin of inexactitude in the duration of centrifugalization, that so easily creeps into the routine performance of automatic work of this sort.

The employment of an anticoagulant in powdered form in hematocrit determinations is preferable in many ways to the use of solutions, provided the anticoagulant is so powerful as to be required in infinitesimal amount. Hirudin appears to answer this specification, for 0.001 gram of the substance is sufficient to inhibit coagulation in 10 c.c. of blood. The phosphatid, heparin, extracted from the liver by Howell[12] also exerts a very powerful anticoagulant effect, and its use in hematocrit tests has already been suggested by Mason.[13] Very obviously these substances, if used in minimal amounts in the blood specimen, would not affect appreciably the osmotic pressure of the plasma by increasing the salt content, but it is not certain that they may not

have such an effect by virtue of their other powerful characteristics. No one has determined this point experimentally. At present the principle obstacle to the employment of these agents with the hematocrit is their scarcity on the market, although it is now possible to obtain heparin in standard preparation.

The hematocrit may be used for the purpose of determining corpuscle fragility. As is graphically represented in Chart 2, with a decrease in the concentration of the surrounding fluid, the red corpuscles increase in size until the limit of distensibility is reached, when rupture takes place (laking), and corpuscle volume drops almost immediately to zero. The starting point of rupture is indicated quite precisely in the curve at 0.72 per cent of sodium oxalate solution. The determination of erythrocyte fragility by this method should be simple to carry out in routine work and of greater convenience than the double value commonly used as an index. With three or four stock solutions of strengths lying within the probable zone of corpuscle rupture and with as many hematocrit pipettes, a single centrifugalization would suffice for the estimation of corpuscle fragility upon an individual. Such a test was used some time ago by Hamburger.[14]

SUMMARY

It is believed that the hematocrit has not attained the degree of popularity as a clinical test that is justified by the significance of the information it reveals, and that the comparative obscurity into which the method has fallen is due largely to inadequacy from the standpoints of facility and accuracy.

Description is given of a new form of hematocrit and of the method of its use. Also, the principles underlying the operation of the hematocrit are critically discussed and put to experimental test, for the purpose of laying down specifications for the use of the instrument which produce reliable results and at the same time remain within the bounds of practicality.

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A CONVENIENT APPARATUS FOR THE DETERMINATION OF LOW CONCENTRATIONS OF CHLORINE IN CHLORINE-AIR MIXTURES*

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RECENTLY an interesting paper† has appeared on the successful use of chlorine as a cure for colds, whooping cough, influenza, and other respiratory diseases in which the infection is located on the surface of the mucous membranes of the respiratory passages. In the use of this agent it is necessary to know, within fairly narrow limits, the chlorine content of the air-gas mixtures breathed by the patients. A simple apparatus, easy of manipulation and capable of accurately measuring the chlorine in dilutions of the order of a few parts (by volume) per million parts of air is of prime importance. Such an apparatus is described in the present paper with the thought that it may be of some assistance to physicians who may wish to try the chlorine treatment for certain respiratory diseases and who may not have access to more elaborate and expensive apparatus. Even when the simple portable apparatus described by Vedder and Sawyer† is used, it would seem advisable to check by chemical analysis the chlorine concentration from time to time; not only on account of possible variations in the amount of gas released by this apparatus, but also on account of the fact that the amount of chlorine lost by adsorption, diffusion, etc., will vary in different rooms. The chemical analysis, the present writer employed, is one of the standard methods for the determination of chlorine and there is nothing novel claimed for the apparatus. The apparatus is, however, simple in construction, easy to manipulate, and is capable of accurate measurement of chlorine in very low concentrations in air. It has been used in some experimental work (not with chlorine as a therapeutic agent) where it was necessary to determine accurately the chlorine content of air containing only a few parts (by volume) of chlorine per million parts of air. Since it was not practical to make the room even approximately air-tight and also since chlorine is rapidly adsorbed by the walls and various objects in the room, it was necessary to make frequent determination of the chlorine concentration. For these experiments the following apparatus and procedure were used successfully.

APPARATUS AND PROCEDURE

An aspirator bottle (2-liters capacity), (Fig. 1) is filled to the mark *C* with water. Forty or fifty c.c. of 4 per cent potassium iodide solution is put in vessel *A* and the screw pinchcock *E* opened and adjusted to a rate of flow of about one liter per minute. The chlorine-air mixture is thus drawn into the potassium iodide solution where the chlorine liberates an equivalent amount of iodine

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†Vedder and Sawyer: Jour. Am. Med. Assn., 1924, lxxxii, 764.

which is soluble in the large excess of iodide solution. As soon as the water has reached the two-liter mark *D*, pinchcock *E* is closed and the absorption vessel *A* is transferred to the receiving bottle. The positions of the two bottles are then interchanged so that the receiving bottle now becomes the aspirator bottle, and vice versa, and the pinchcock *E* again opened. This procedure is followed until the desired volume of air-gas mixture has been drawn through vessel *A*, or if preferred, a large (ten-liter) aspirator may be used and thus avoid the interchanging of vessels. When a sufficient sample (say ten liters) of air-gas mixture

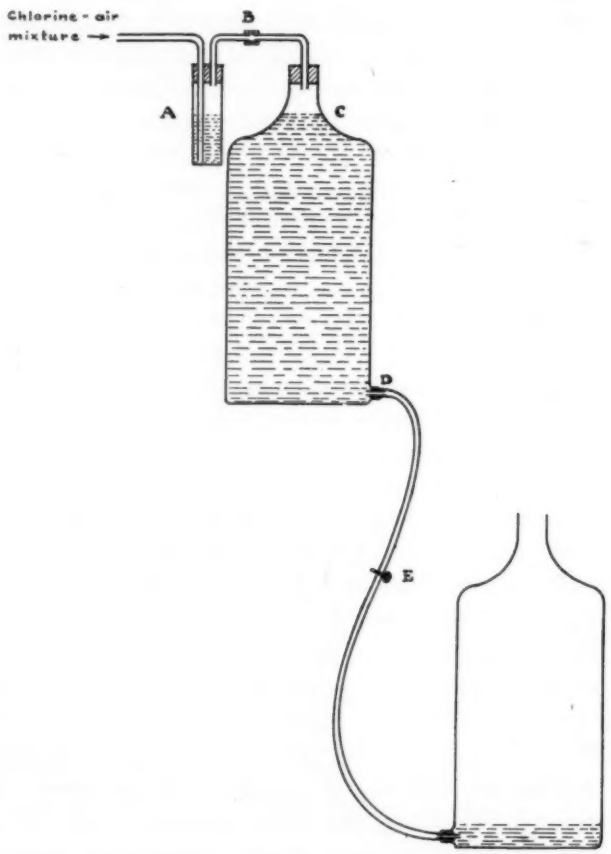

Fig. 1.

has been taken, vessel *A* is disconnected at *B* and the free iodine titrated with standard sodium thiosulphate solution, using starch suspension as an indicator. The results are calculated to parts (by volume) per million at 20° C. and 760 mm. pressure.

SOLUTIONS

1. Sodium thiosulphate solution (0.00166 N). Dissolve about 0.3 gm. of sodium thiosulphate, $Na_2S_2O_3.5H_2O$, in a liter of freshly boiled distilled water and standardize against 0.0050 N iodine solution. Dilute the thiosulphate solution until the required strength of 0.00166 N is obtained.

2. Potassium iodide solution (4 per cent). Dissolve 40 gm. of potassium iodide in a liter of water.

3. Starch suspension. Add about 3 gm. of starch to one-half liter of hot water and boil for one hour. Filter while hot and keep in a bottle stoppered with a cotton plug.

CALCULATIONS

The standard thiosulphate solution is made of such a strength that each c.c. is equivalent to 20 parts per million (p. p. m.) of chlorine at 20° C. and 760 mm. in a one-liter sample. Then by taking a ten-liter sample, each c.c. of thiosulphate solution will be equivalent to two p. p. m. of chlorine. The method of calculation is as follows:

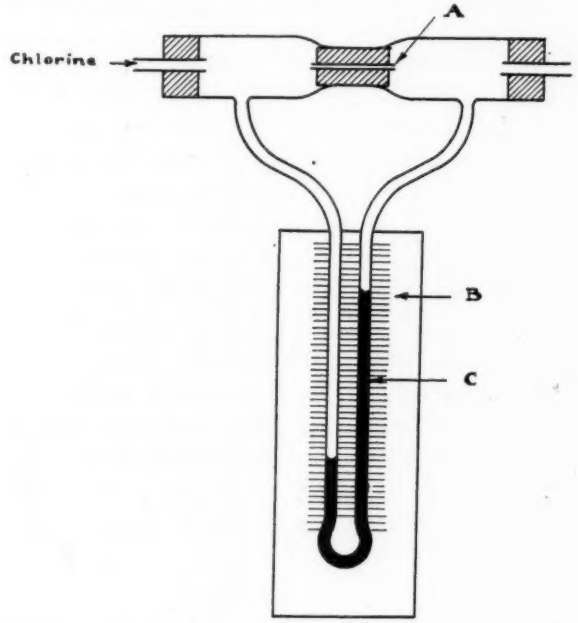

Fig. 2.—*A*, Small glass capillary tube through a rubber stopper; *B*, Millimeter scale; *C*, Water or oil manometer.

$$1 \text{ equivalent of chlorine} = \frac{24.04}{2} = 12.02 \text{ liters at } 20° \text{ C. and } 760 \text{ mm.}$$

1 liter of normal sodium thiosulphate = 12.02 liters of chlorine at 20° C. and 760 mm.
1 c.c. of 0.01 N sodium thiosulphate = 0.1202 c.c. of chlorine at 20° C. and 760 mm.
In a liter sample, 1 c.c. 0.01 N sodium thiosulphate = 120.2 p.p.m.
1 c.c. 0.00832 N sodium thiosulphate = 100 p.p.m. in a liter sample at 20° C. and 760 mm.
1 c.c. 0.00166 N sodium thiosulphate = 20 p.p.m. in a liter sample at 20° C. and 760 mm.
For a sample of X liters,

$$1 \text{ c.c. } 0.00166 \text{ N sodium thiosulphate} = \frac{20}{X} \text{ p.p.m.}$$

10 p.p.m. chlorine = 0.03 mg. chlorine per liter.

Obtaining the Chlorine Concentration.—The desired concentration of chlorine may conveniently be obtained from a cylinder of liquid chlorine to the valve of which is attached, by means of a short rubber tube, a glass capillary flow

meter (Fig. 2). The flow meter may be calibrated (see below) so that under a given head (preferably not less than 10 cm. of water) it delivers 50 c.c. of chlorine per minute. This would mean that a three-minute flow would be sufficient to give a chlorine concentration of approximately 5 p. p. m. (0.015 mg. per liter) in a room of about 1000 cubic feet capacity. To insure a rapid and even distribution of the gas, an electric fan should be placed near the outlet of the flow meter. Having introduced into the room the amount of chlorine to give approximately the desired concentration, i. e., 5 p. p. m., the exact chlorine con-

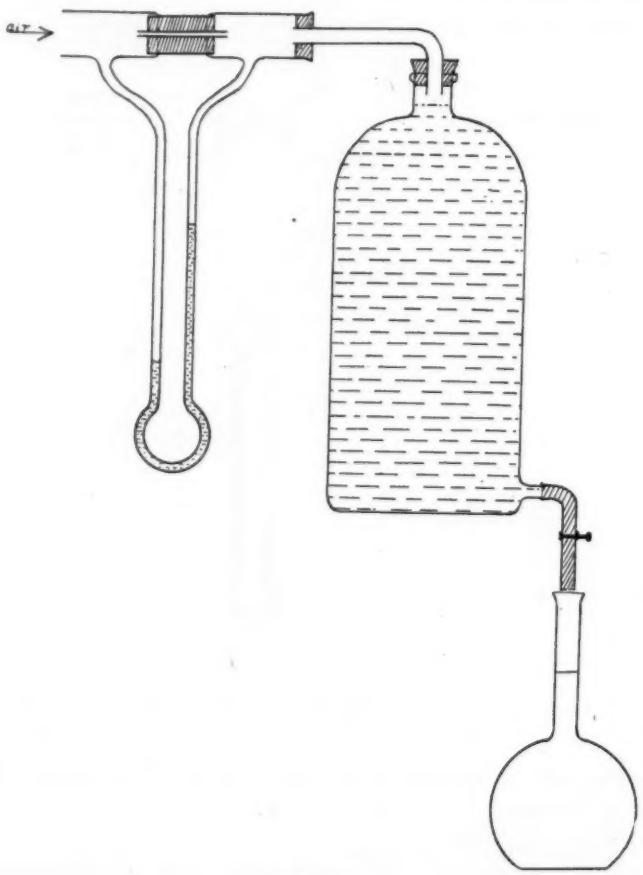

Fig 3.

centration is then determined by drawing a ten-liter sample of the air-gas mixture in the manner described above, under Apparatus and Procedure, and titrating the liberated iodine with standard sodium thiosulphate solution. As noted in the accompanying table, a titer of 2.5 c.c. of 0.00166 N sodium thiosulphate solution is equivalent to a chlorine concentration of five parts (by volume) per million at 20° C. and 760 mm. of pressure.

Calibration of Flow Meter.—The capillary tube *A* (Fig. 2), should be about 50 mm. long and should have a radius approximately 0.2 mm. The flow meter may be calibrated directly against pure chlorine but more conveniently against air, making the proper allowance for the difference in the viscosity of air and

TABLE OF EQUIVALENT DATA FOR A 10-LITER SAMPLE

C.C. 0.00166 N $Na_2S_2O_3$ USED	CHLORINE P.P.M. (VOLUME) AT 20° C. AND 760 MM.	MILLIGRAM CHLORINE PER LITER	REMARKS
0.5	1	0.003	
1.0	2	0.006	
1.5	3	0.009	Odor distinct
2.0	4	0.012	
2.5	5	0.015	Desired concentration
3.0	6	0.018	
3.5	7	0.021	Slight irritation of throat
4.0	8	0.024	
4.5	9	0.027	
5.0	10	0.030	

that of chlorine. To calibrate against air, simply connect the flow meter to an aspirator bottle (as shown in Fig. 3) and find the difference in water levels in the two arms of the manometer that corresponds to an air flow of 40 c.c. per minute. The volume of air being drawn through the meter in any given length of time is obtained by noting with a stop watch the time required to fill a volumetric flask of, say, 500 c.c. capacity. Suppose the time is found to be 12.5 minutes and the difference in the height of the water in the two arms of the manometer is 10 cm.,—this would mean that with a 10 cm. head of water the flow meter will deliver 40 c.c. of air per minute or approximately 50 c.c. of chlorine per minute, since the ratio of the viscosity (at 20° C.) of air to that of chlorine is 1:1.25. Several readings with the stop watch should be made and the average taken. In each case, carefully adjust the flow of water from the aspirator bottle before putting the volumetric flask in place. This calibration is sufficiently accurate, since the concentration is finally determined by actual analysis of the air-gas mixture in the room.

AIDS TO URINALYSIS IN A LARGE HOSPITAL*

By C. Pons, M.D., and E. B. Krumbhaar, M.D., Ph.D., Philadelphia, Pa.

TO promote the better examination of urine in the central laboratory of a large hospital, we have found the following devices to be practical and useful.

I. *Urine Tubes and Transportation Rack:* Special urine tubes are provided, made of heavy glass, with spout and blunt conical bottom. They are 7½ inches long by 1¼ inches wide (outside) and have an approximate capacity of 90 to 100 ml. This promotes sedimentation, and permits convenient floating of a urinometer, with considerable saving of carriage space over the old sedimentation glasses. The transportation rack (Fig. 1) has 16 numbered apertures, corresponding to the numbered tubes, thus reducing the liability of confusion. The bottom of the tray contains felt or rubber matting to minimize breakage. Loaded

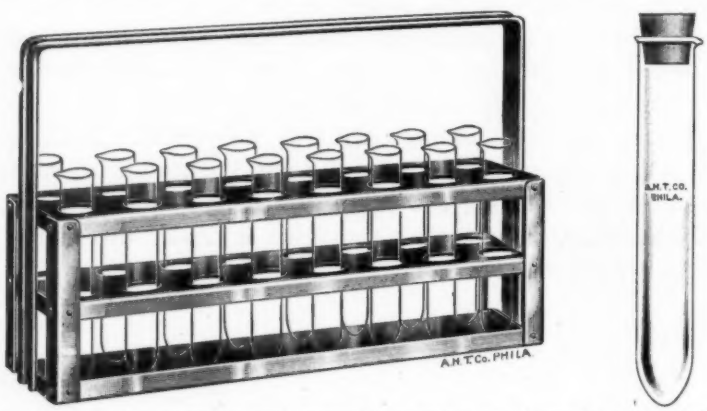

Fig. 1.—Tray for carrying and examining urine, with special urine tube.

racks are supplied to orderlies as the racks filled with specimens are brought in, each tube containing a few cubic centimeters of a 4 per cent solution of formaldehyde (i.e., same as used for tissue fixation, to prevent alkaline decomposition of urine) and securely corked. The laboratory slip is folded, wrapped about the tube and held with a metal clip. The advantages of this system are found to be that, (1) a uniform method of collection is provided; (2) curtailment of handling of the specimen in the laboratory; (3) decomposition is minimized; (4) centrifugation is as a rule unnecessary; and (4) the caliber and capacity of the test tube permits the specific gravity to be taken therein.

II. *Albumin Test:* For the heat and acetic acid test for albumin, Kolmer's wire rack is used, and sixteen ordinary medium sized test tubes of thin glass,

*From the Laboratory of Clinical Pathology of the Philadelphia General Hospital, Philadelphia, Pa.

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Received for publication, February 12, 1925.

placed in close apposition in a row of eight each. These are two-thirds filled with urine and heated simultaneously with a fishtail burner, which covers five test tubes at a time. This not only causes a considerable saving of time, but minimizes boiling over and breakage. The rest of the test is performed in the usual manner. To promote the consistent reading by different internes, of the amounts of albumin present, Dr. Karr of our biochemical laboratory has prepared a permanent series of tubes corresponding to "faint trace," "trace," etc., up to "heavy cloud." These consist of sterile, formalinized gelatin, containing increasing amounts of albumin in the upper half of the tube and hermetically sealed. Against a black background these give a very fair comparison with the urine being tested. Lately we have adopted Purdy's[1] method for the detection of albumin, except that instead of using a 50 per cent solution of acetic acid,

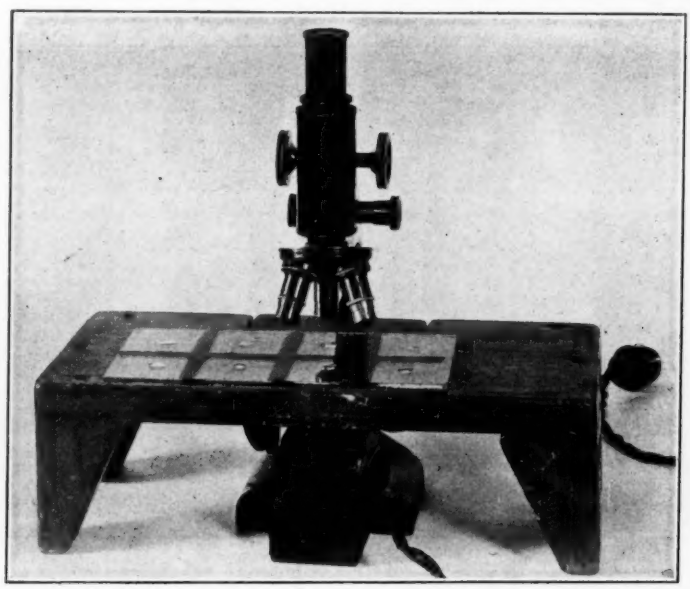

Fig. 2.—Special microscope stand and mammoth slide for examination of urine sediments.

we use a 25 per cent solution. We have found this test as sensitive as the heat and acetic acid test and practically no more trouble. Mucin, which is present in many urines, and which gives a positive test by other methods, is held in solution by the saturated sodium chloride, as used in Purdy's method. While we agree it is better to filter all urines for any aubumin test, with this method necessary filtration is cut to a minimum.

III. *Qualitative Sugar:* Benedict's solution is used exclusively. Forty-eight tests are done simultaneously by immersing a Kolmer rack in a boiling water-bath for two and one-half minutes. The timing is important, as longer boiling gives some fallacious reductions.

IV. *Quantitative Sugar:* For the last six months we have used Sumner's[2] dinitrosalicylic acid colorimetric method with the following advantages over the Benedict method in our estimation. Under our conditions, internes' errors are less frequent. It is not as expensive and there are fewer steps and

no calculations. It has the further advantage that any number of tests can be done simultaneously. Practically all the solutions used are placed in aspirator bottles to save time.

V. *Microscopic Examination:* To save time, without loss of accuracy, several pieces of ordinary windowpane glass, about 4 by 8 inches are prepared. These are divided by heavily painted lines into eight compartments each of which is about 2 inches square. The usual microscope stage is extended by a wooden table (as in Fig. 2), thus permitting the free movement of the mammoth slide under the objective. The urine from the conical ends of the original containers is pipetted off, eight at a time, a washed pipette being used for each specimen. As the pipette is simply a piece of tubing drawn to a blunt point a large number can always be available. Unless the high power is needed in an exceptional case, cover glasses are not needed, and the eight sediments can be examined long be-

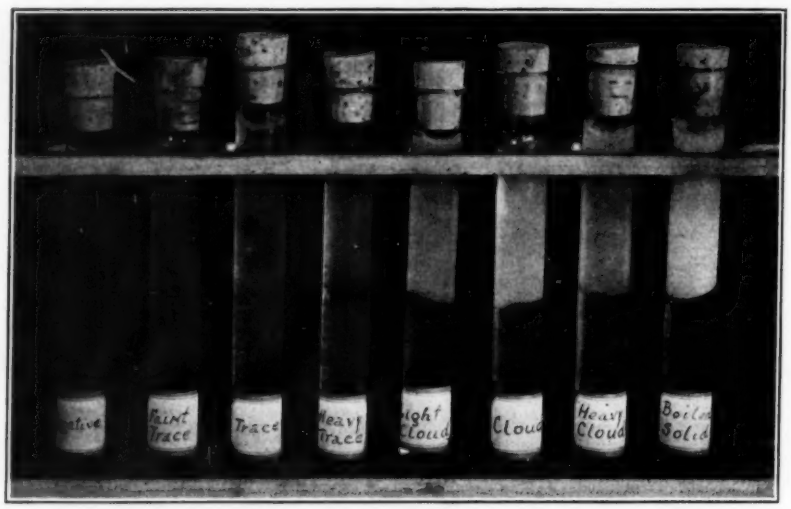

Fig. 3.—Permanent albumin standards for urinalysis.

fore any harmful drying has taken place. The two inch square provides an extra large area for the accommodation of a large sample of urine.

Mr. Thomas Smull has aided us in the carrying out of these ideas.

A rotating rubber stamp, with a capacity of twelve letters, is used for the interne's signature. With over 100 urines daily this constitutes a considerable saving of time.

SUMMARY

1. Improved forms of urine collection tubes and transportation racks are described.

2. A quick improved method of performing the heat test for albumin is given, with permanent standards of comparison.

3. Improved qualitative and quantitative sugar methods are discussed.

4. A device for the quicker microscopic examination of urinary sediments, without loss of accuracy, is also described.

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DEVICE FOR SAVING SUPERNATANT FLUIDS*

By Fred A. Wiggers, Toledo, Ohio

IT is essential in many laboratory procedures to save supernatant fluids. At times this entails the laborious task of pipetting off the fluid by hand. Especially is this true when the volumes are large. There has been no available apparatus in the market for accomplishing this procedure without losing the fluid into the drain pipes. Most of the mechanical decanting has been with the suction pump. With this method it is evident that the supernatant fluids are lost.

With the idea of saving much hand pipetting I devised, some years ago, a simple apparatus to collect supernatant fluids. This was accomplished by converting an ordinary straight-sided separatory funnel into a receiving funnel. By elaborating this scheme somewhat, a funnel was obtained that seems to meet all requirements. See Fig. 1.

In detail it is a separatory funnel with a closed roof, an inlet pipette for the intake of fluids, and a tube leading to the suction pump. In principle, the pump pulls a vacuum in the funnel, and this vacuum, in turn, pulls the fluid into the funnel. The entire operation can be aseptically carried out and the fluid preserved in a sterile condition. The funnel may be used for any class of work.

DISTILLING APPARATUS

Every laboratory has had the task of distilling and redistilling volatile fluids. Any such fluids are dangerous and great caution is required in distilling them. In most laboratories the usual procedure has been to distill them over from a flask resting in a water-bath. Such methods are cumbersome and dangerous.

Several years ago, I had a copper jacket arranged about a Florence flask and some water placed between the two. This method was not entirely satisfactory where an all-glass still was needed, and so the double-flask still (Fig. 2) was planned. This is simply a large flask completely incasing a smaller one, leaving a space for water between the two. In practice, water is allowed to enter through the pipette into the space between the flasks. This water is heated and the temperature regulated by a thermometer thrust into a suitable opening provided for it. Toward the top of the neck of the larger flask is a pet cock with a glass stopper to act as a safety valve for steam.

*From the Pathologic Laboratories, Toledo Hospital, Toledo, Ohio.
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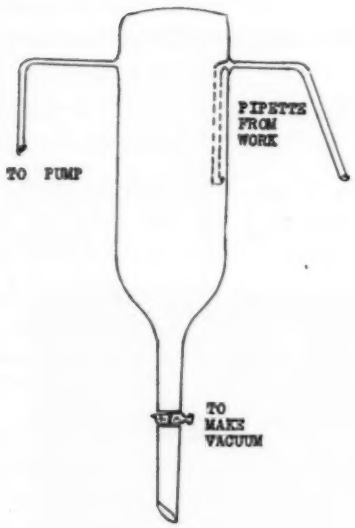

Fig. 1.

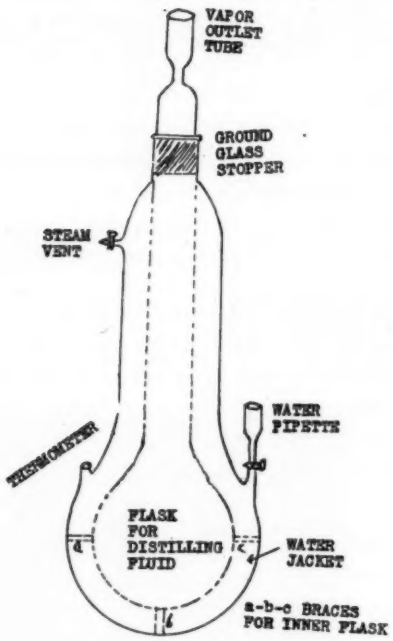

Fig. 2.

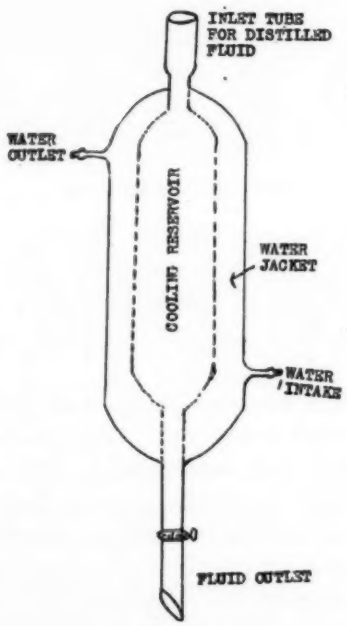

Fig. 3.

The neck of the inner flask rises above that of the larger one and is provided with an outlet tube for the vapor to be passed to the condenser. This outlet tube fits like a ground-glass stopper and the whole apparatus is easily cared for and kept clean.

COOLING RESERVOIR

In the redistillation of highly volatile fluids such as hydrogen peroxide, ether, gasoline, etc., there is always great danger of explosions. The apparatus pictured in Fig. 3 offers a solution for controlling the vaporization of the more highly volatile substances.

In principle, the apparatus is designed so as to confine the distilled fluid in a reservoir which is incased with an outer jacket through which running water flows. It is similar in this respect to that of a condenser. This running water has the effect of reducing the temperature enough so that volatilization is materially diminished. In practice the condensed vapor is received from the condenser and allowed to enter the reservoir. The connection between the condenser and the reservoir is made air-tight. Water is circulated through the outer jacket. When distillation is completed the apparatus is removed and the fluid allowed to flow out through the drawn-out stem below. A glass stopcock is, of course, provided to enable this removal. The entire apparatus is of glass.

No dimensions or capacities are given for either apparatus for the obvious reason that different sizes and capacities are needed for different laboratories.

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EDITORIALS

Psychology and Anesthesia

NO group of drugs is more deserving of the grateful appreciation of all mankind than are those which are used to produce anesthesia or analgesia. A peculiar psychologic interest attaches to these substances in more ways than one. Many years ago Professor Jones had himself anesthetized with ether to the stage of unconsciousness seven different times in order that he might study at first hand, and in a strictly scientific manner, the strikingly interesting phenomena which ether is capable of producing in those who inhale its vapors. The experiments were carried out in a psychologic laboratory and special equipment and devices were provided so that observations could be made and recorded constantly throughout the course of the experiments. Trained attendants assisted Dr. Jones and made the necessary objective observations while recording the subjective sensations as they were described by Dr. Jones.

Here a phase of anesthesia which receives but little, if any, attention from the average medical man was studied and analyzed. The gradual fading away

of one's awareness of all earthly things and conditions was strikingly illustrated. The experience is somewhat similar to that of one who watches the receding shoreline as he stands on the deck of a vessel which is rapidly speeding out in the mists and fog of a rolling sea. One by one the special senses give way, each from moment to moment adding something to, or subtracting something from, one's mental contact with the outside world, with his own physical state or with his own inner consciousness. It is probable that nearly all patients, who are fully anesthetized, go through some such experience as this. But the conditioning circumstances under which the anesthetic is given, the mental character and attitude of the patient, and his previous notions of what should happen, have a great deal to do with his psychologic reaction to the effects of the anesthetic.

Dr. Jones, however, brought out an interesting feature of his experience,—a phase which apparently must have been reached a little while before total unconsciousness supervened. In this phase, which might be prolonged by proper administration of the anesthetic, all sensation was lost. Every special sense was cut off entirely from its connection with the inner consciousness. The mind, however, could still function and found itself freed from the usual restraints of the realities of life. As Professor Jones put it, he was in "the position of a disembodied spirit." Time and space no longer offered any obstacles, while physical infirmities and earthly longings had suddenly ceased to exist. Like a butterfly his soul could flit from star to star. What a splendid reality of unrealities;—what a satisfying physical demonstration of a state, which previously could have had only an imaginary existence, this experience might have been for some of the old philosophers in their attempts to found philosophic systems based on some such conception of the human mind as this. And perhaps the founders of some of our modern semireligious, semimedical systems might have profited by a study of some of the mental phenomena which anesthetics are capable of calling forth.

Let us carry the experiment a little farther. It is entirely possible in the laboratory to administer an anesthetic to an animal which may be presumed to pass through an experience resembling that produced in man. But if the anesthetic is pushed, by and by we reach a point where the respiration has stopped and the heart has just ceased to beat. So far as the animal is concerned it is as dead as it will ever be. Exactly this same thing may, and indeed frequently has happened in the case of man. However, under these circumstances, it is usually possible by the use of appropriate means promptly to revive the animal. An injection of adrenalin solution directly into the cavity of the left ventricle, artificial respiration carried out by means of a suitable mechanical device, and careful massage of the heart, may usually be counted on to readily restore the animal. Obviously, with similar treatment, the same thing would happen in man. Here we have Professor Jones' experiment carried to the last degree. In those few moments when the man has ceased to breathe and his heart no longer beats, has he indeed become a disembodied spirit or is he still a living soul? When only three drops of chloroform, or a cubic centimeter of adrenalin solution, stand between man's soul and a fathomless future on the one hand, or a hard cold world on the other, just what may his status be presumed to be? In

these days when so many people are trying to find out whether evolution is or is not evolution, we should like to propound to these same people, the question as to what is the status of the soul of a man who is no longer living but is not yet dead.

It is interesting to note that there are styles and fashions in anesthesia the same as there are styles in clothes. Forty and fifty years ago chloroform was very popular. Most of the young surgeons who got their training in those days clung to chloroform faithfully until they ceased to operate or else were literally forced to give up chloroform by a younger generation of surgeons. Then ether came in style and finally nitrous oxide with oxygen. But the styles changed slowly and were in reality very good indices of the rate at which one generation of surgeons can shove the preceding one out of the field of operations.

But now we have another of these psychologic, style-changing contests before us. Ethylene has but recently been introduced by Luckhardt as a new anesthetic. It has a large field of usefulness before it. For many types of operations, and in a great variety of conditions, it undoubtedly possesses advantages over any other general anesthetic now in extensive use. Does this mean that ethylene will be widely and rapidly adopted in the surgical clinics of the land? By no means. If we may draw any conclusions from the development of anesthesia in the past we may well conclude that only by another generation will ethylene be utilized to the fullest extent of its possibilities. And while everyone recognizes the danger of its explosibility still it now seems probable that this danger will soon be reduced to a minimum. It is not this feature that will delay the introduction of ethylene, but it is in reality the psychologic limitations which God has prescribed for surgeons (anesthetists?), and perhaps for a number of others who do, or should, use anesthesia in their work. But there is only one possibility that can remove ethylene from the sphere of its future usefulness, and that is the possibility that some still more satisfactory anesthetic may be found to take its place.

—*D. E. J.*

The Diagnostic Significance of Red Blood Cell Volume

THE estimation of the size of the red blood cell is now recognized as a most valuable diagnostic and prognostic measure in the study of anemia. On the basis of differences in size, classifications of the anemias, which represent fundamental cell properties, are possible. "Size" may be expressed in terms either of diameters or of true volume. It is preferable to determine the actual cell volume, since the thickness of cells is as variable as the diameter or even more so. One not uncommonly finds cases of pernicious anemia in which there is a marked increase in average cell volume without significant change in diameter as a result of the marked increase in thickness.

The first determinations of cell volume were made by Hedin, in 1893,[1] using the hematocrit of Blix. Herz,[2] in a series of cases, counted the red cells simultaneously with the determination of the volume of cells in a known amount of blood. He described a factor represented as the "mean volume of a single cell"

which he found quite variable in disease. To Capps,[3] however, is due the credit for making the first accurate study of average cell volume and applying clinically the facts so learned. Capps introduced the term "volume index" which represents the volume of the average red blood cell in terms of normal cells and affords a simple means of comparison.

In estimating the volume of red cells, Capps employed the small hematocrit tubes of Blix, which give only the relative mass. A simpler and perhaps more accurate method[4] is to centrifuge 10 c.c. of blood mixed with 2 c.c. of isotonic sodium oxalate (1.6 per cent). In this procedure the absolute volume of the cells is obtained. Van Allen[5] has recently suggested a modified micro tube which necessitates no special apparatus other than the tube, but also gives only relative readings. In calculating the volume index only a red cell count is necessary in addition to the relative or absolute cell mass. Capps pointed out that in pernicious anemia the volume index is greater than 1.00, while in secondary anemia it is 1.00 or less. This has been amply confirmed by other observers.

Price-Jones[6] and other English observers have determined the "size" of the red cells in terms of diameter. Five hundred cells on a stained film are drawn in outline, magnified a thousand times, and measured with a millimeter scale up to 0.5 mm. The mean diameter of the five hundred cells is taken to represent the average diameter of the blood cells of the person tested. Pijper[7] has suggested an optical method for determining the cell diameters utilizing the blood film as a diffraction grating. Such procedures are, however, much more laborious and time-consuming than the method used in the determination of the volume index. They fail to show also the true size in these cases in which there is a marked increase in thickness with consequently much greater cell volume but relatively little change in diameter.

The size of the average cell is remarkably constant. The average volume index is always approximately 1.00 in normal adults. The normal average mean diameter is 7.24 micra.[6] In absolute terms the actual average cell volume is 9.6 by 10^{-11} c.c.[8]

The English observers agree that the average mean diameter is always much increased in pernicious anemia. Passey[9] records similar results in sprue, Dibothriocephalus anemia, and in anemia produced experimentally in rabbits by the injection of the toxin of hemolytic streptococci. Any deviation from the normal in other secondary anemias is manifested as a decrease in size. Such differences suggest the separation of anemias into megalocytic and nonmegalocytic groups, which represent fundamental cell differences. There is much to suggest that the volume increase in the group so affected represents the action of a toxin common to the various clinical conditions in which it occurs.

Price-Jones points out that the red cell diameters are independent of the degree of anemia in pernicious anemia. The increase in size may be apparent at the onset when the degree of anemia is slight. This fact has been emphasized also in studies of volume index. In fact, in pernicious anemia the volume index may be much greater in the early stages when the count is relatively high than in the later stages when the count is low, thus indicating its value as an early diagnostic test. The conditions other than pernicious anemia in which the

average cell size is increased are infrequently observed and can be readily differentiated clinically. A definite increase is presumptive evidence of pernicious anemia.

Knowledge of the cell volume is also of the greatest value in the prognosis of secondary anemia. Increase in cell volume must precede the increase in the hemoglobin content. Given two patients with low hemoglobin, improvement is necessarily more rapid in the one having the average cell volume nearest normal.

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—*R. L. H.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*Practical Anaesthetics**

THIS book is a classic in brevity, conciseness, and accurate information. The details and pertinent facts of general, local, infiltration, regional, intrasacral, spinal, and splanchnic anesthesia and analgesia are all "practically" covered. A well-written history is condensed into six pages.

"Ethanesal, a preparation of mixed ketones dissolved in purified ether to the strength of 5 per cent" is urged to take the place of ether, and it should if the authors are correct. This opinion is based upon the practical effects of ethanesal as observed by Boyle and his associates in over 10,000 cases: "Less taste and smell, consequently less irritation, and quieter respiration, more analgesic, fewer after effects, as vomiting."

In reviewing the local anesthetics in use, it is stated that, "On the whole, novocaine appears to be the best combination of efficiency and low toxicity."

The book seems especially adapted for the busy surgeon in addition to being a valuable handbook for the anesthetist.

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*Practical Anaesthetics. By H. Edmund, G. Boyle and C. Langton Hewer. 187 pages with 45 illustrations. Third edition. Oxford Medical Publication, London, Henry Frowde and Hodder and Stoughton.

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